THE
JOURNAL OF
COMPARATIVE PATHOLOGY
AND
THERAPEUTICS.

Vol. LI.—No. 2. JUNE 30th, 1938 PRICE 4s.

ENZOOTIC PNEUMONIA IN SHEEP

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THE object of this communication is to record the result of an investigation on ovine pneumonia which was undertaken jointly by the authors. The majority of the outbreaks which were studied occurred in North Wales where the disease showed a fairly regular seasonal incidence, thus providing an opportunity for the collection of suitable material. As the investigation progressed cases of a similar nature were also observed in other areas.

OCCURRENCE AND SYMPTOMS.

Although sporadic outbreaks of what appeared to be an infectious pneumonia among sheep had been encountered in North Wales from 1926 onwards, it was not until the spring of 1935 that the disease occurred in enzootic form. In that season the flocks on several farms in four quite separate districts became affected and similar outbreaks occurred in 1936. During 1935 and 1936 the disease was also seen in single flocks at three other centres, in one instance in the late autumn. There are good reasons for believing, on epizootiological grounds, that the condition observed in all these flocks was identical and this view is supported by the symptoms, *post-mortem* appearances and type of organism obtained from the lung tissues.

In some outbreaks the first indication of the presence of disease was the discovery of a sheep which was obviously ill, but more usually excessive coughing after rounding up the flock attracted attention a day or two before serious symptoms were noted. On close inspection a mucoid discharge from the nose was seen in the more obviously affected sheep, but spasms of convulsive coughing were more

characteristic. The temperature appeared to be little elevated in the early stages and it was only later that affected animals showed loss of condition, a dryness of the wool and a tendency to isolate themselves from the rest of the flock. In advanced cases there were obvious signs of distress, the animal standing with head hanging and legs spread apart, the picture of dejection. Respirations were of a shallow, abdominal type, the temperature although generally much elevated, was occasionally subnormal and there was a copious nasal discharge, often accompanied with some discharge from the eye. The period between the detection of symptoms and death varied widely. Some ewes, showing only violent spasms of coughing, died within twelve hours ; others gradually sank in condition and were killed or died five to seven days later. The natural recovery rate is unknown but must be considerable. The actual losses from death rarely exceeded 15 per cent. of the flock, despite the fact that the violent coughing which was evidenced when the animals were rounded up suggested that every sheep was affected. In the majority of flocks the loss of condition was a source of great financial loss among ewes with young lambs at foot.

It was remarkable that in North Wales the condition was generally confined to mature animals, including rams as well as ewes, despite the fact that some flocks contained young lambs from one week to three weeks old. In one flock, however, a few yearling sheep did contract the disease. There was some suggestion that ewes in low condition were particularly susceptible, but this appeared to be fallacious and largely arose because of the loss of condition which had taken place before the symptoms became obvious. Ewes in good lambing condition, and even high conditioned rams being prepared for show, died of the disease in its more acute form.

It has often been possible to associate some violent change in climatic conditions with the appearance of outbreaks of disease ; for example, one of the most extensive followed the short heat wave of early May, 1935, and the succeeding period of cold east winds. More usually, some crowding together of the sheep, housing, washing or dipping, penning, travelling on foot or in trucks or a combination of these circumstances has preceded the appearance of the disease in the flocks. An isolated outbreak which occurred in 1926 had a particularly interesting history. Two brothers bought 100 ewes and walked them in hot weather along dusty by-roads to the farm of one brother where 50 were drawn at random. The remainder proceeded towards the second brother's farm some six miles away but, through delays *en route*, were put into the stable of a roadside farm and remained there overnight. Eight days later two of these sheep died of pneumonia and in the succeeding days practically all the others showed symptoms of the disease, the total number of deaths being nine. There was no evidence of disease on the farm from which the ewes were purchased or on that on which they spent the night, nor was the other half of the purchased sheep affected. In fact,

careful enquiry failed to elicit evidence of the disease on any farm along the route these sheep travelled. It would seem that the housing in the stable was the contributory factor. In an outbreak which occurred in July, 1936, the injection of a vaccine prepared from a strain of the organism described later in this article appeared beneficial, but when the sheep were mixed with others and moved to new premises by motor wagon in the following September a serious recurrence caused many deaths. It is not known whether it was the vaccinated or unvaccinated sheep which suffered, but it seemed evident that the crowding together of the flock was a major factor in the reappearance of the disease. The possibility that larvae of *Oestrus ovis* might play some part in the production of the disease was not overlooked, but in only one flock was occasional infestation with this grub encountered. Furthermore, sheep in certain composite flocks, some having spent the previous summer on widely separate farms, were affected to a similar extent. The possible influence of nematode parasites in the lung tissue was also considered but no direct evidence was obtained that they played any significant part as contributory factors in the etiology of the condition.

Particular attention was paid to the presence of parasites in the outbreaks which occurred in other areas in which the circumstances were somewhat different from those obtaining in North Wales and were as follows :—

Flock 1.—This was a flock of ewes in which there was no previous history of pneumonia. A number of them had been purchased in the autumn but had remained healthy up to and during the outbreak which occurred in December. Several ewes, all of which had been bred by the owner, died. They were in moderate condition and were free from lung parasites. The *post-mortem* examinations showed a severe pneumonia, affecting both lungs, accompanied by pleurisy.

Flock 2.—A consignment of sheep was sent by rail from Scotland to supplement this flock. The animals were in good health on arrival and remained bright for four to five days. During the succeeding period, however, several became ill and died. The losses were confined to the bought-in sheep. A *post-mortem* examination showed extensive congestion of the lungs with areas of consolidation. Numerous parasites were observed in the pneumonic areas, but whilst the pulmonary changes might have been partly attributable to their action it was obvious that the consolidation was much more extensive than is the case in uncomplicated parasitic infestations.

Flock 3.—The owner of this flock, which was self-contained, had experienced quite heavy losses in his lambs and ewes in previous years, but the veterinary practitioner was satisfied that the deaths had been due to simple parasitic infestations. On this occasion, however, the mortality was much higher and the animals, including some of the lambs, succumbed after a short period of illness with symptoms of severe pulmonary involvement. In each instance a *post-mortem* examination showed an extensive broncho-pneumonia, but caseous lesions were also present in the lungs of some of the animals. The majority were suffering from severe parasitic gastro-enteritis.

Flock 4.—The feature of this outbreak was the sudden onset of symptoms in a flock of fat lambs at the beginning of July. The flock was self-contained and had been free from infection of a pneumonic type. The disease was confined to the lambs, many of which died after a period of illness of not more than 48 hours. *Post-mortem* examinations showed extensive congestion of both lungs with consolidation of large areas (up to one-third of lung tissue). The lungs were free from parasites.

It will thus be seen that two of the outbreaks occurred in mid-winter, one in early spring and one in summer. In two cases, a heavy infestation with pulmonary and/or gastro-intestinal parasites was detected in the affected animals whereas in the others the sheep were free from this complication. In one instance, the disease broke out in bought-in animals to which it was confined ; in two, there was no history of an introduction of fresh animals. The significance of these observations in relation to the etiological importance of the bacteria found in the lungs is somewhat striking and will be discussed at greater length elsewhere.

Post-mortem Appearances.

(*a*) *Gross Lesions.*—The *post-mortem* abnormalities, apart from an appearance which suggested some cloudy swelling of the liver and occasionally of the kidneys, were confined to the thoracic cavity and varied much in type and extent depending on the course of the disease. In the more acute cases both lungs were extensively affected with an acute pneumonia, pleural fluid was present in considerable excess and numerous petechial hæmorrhages were seen in the pleura, pericardium and epicardium. In a few instances the disease proved fatal before much of the lung tissue had become consolidated and the main change then observed was acute and extensive congestion. In chronic cases the pneumonic changes were often restricted to a small part of one or both lungs which showed grey and red areas giving a mottled appearance to the affected tissue. The pleural surface was covered by a layer of fibrin, strands of which stretched between the lungs and the chest wall. In acute cases the lining of the bronchi showed marked inflammation, but this was hardly noticeable in chronic cases. Parasitic invasion of lung tissue occurred in some affected flocks but was absent in others.

(*b*) *Histology.*—The histological appearance of the affected lungs varied from area to area according to the stage of development of the pneumonic process. Early changes took the form of inflammation of the smaller bronchi and acute congestion of the surrounding lung tissue. The capillary vessels of the bronchi were engorged with blood and there was swelling and commencing desquamation of the cells of the mucous membrane. Extravasated red cells and serous fluid were also present in the lumen. A varying degree of cellular infiltration was sometimes observed around the bronchi. The walls of the corresponding alveoli were thickened and stood out prominently owing to intense congestion of the capillary vessels and œdema. Extravasated red cells which were often very abundant were

to be found lying free in the alveoli which, in many cases, were filled with serous fluid. The amount of fibrin formation varied, but it was never a striking feature of the lesion such as may be observed in some cases of true pasteurella infection of lung tissue. As the condition progressed the exudate of the alveoli became highly cellular owing to the presence of numerous mononuclear cells resembling histiocytes in appearance, together with a small proportion of polynuclear leucocytes and catarrhal cells. In some cases parts of the hepatised areas had become necrotic whilst the alveoli surrounding them often showed catarrhal changes. In the later stages the bronchi were filled with cellular exudate and there was much desquamation of their epithelial lining. Where the interlobular septa became involved in the inflammatory process they showed a moderate degree of distension with fluid and cellular elements. The pleura over the hepatised areas was inflamed and showed a fibrinous layer on its surface.

The changes described represent an acute type of inflammation as observed in animals dying after a short period of illness and showing little or no evidence of reparative activity on the part of the lung tissue. All of the cases examined were of this type and although a few showed commencing fibrotic changes it was thought that these were not secondary to the pneumonic process, but had resulted from the presence of worm parasites.

As will be mentioned in a later part of this article, the lesions found in the sheep which were used for transmission experiments were not comparable to those seen in natural cases of the disease. The most striking feature of the former was a pleurisy manifested by a large quantity of fluid in the chest cavity and a thick, gelatinous exudate on the surface of the lungs. The abnormal parts of the lung tissue itself were in a state of collapse. Under the microscope they appeared intensely congested and somewhat œdematous. Inflammatory changes were observed in those animals in which the material was injected directly into the lung tissue but were confined to an area around the point of inoculation, except in one case which was complicated by a secondary infection.

Bacteriology.

In each outbreak it has been possible to obtain one or more fresh carcases or ailing sheep for detailed examination. When the affected flock was located at a distance from the laboratory, suitable apparatus was taken to the farm, specimens obtained and media inoculated under conditions which precluded contamination. Otherwise, the carcase or sheep was transported by car to the laboratory. Material for bacteriological investigation was obtained in this manner from 22 cases in North Wales. From four of these no organism of significance was isolated. Two of them which were sheep slaughtered as very early cases showed no gross lesions on *post-mortem* examination and two were carcases from flocks the subsequent history of which led one to doubt the earlier clinical diagnosis. In 18 cases the

pasteurella-like organism to be described later was isolated usually in pure culture from lung tissue only, media inoculated with other body fluids and tissues remaining sterile. Occasionally putrefaction had commenced and other bacteria, chiefly cocci and anærobic bacilli, contaminated the lung cultures and grew in media inoculated from other sites. Unfortunately, many of the earlier strains of pasteurella died out during the few days of storage before they could be fully investigated, but on simple morphological and cultural characters it appeared that this type of bacterium was consistently present in each typical outbreak. With the exception of the first few cases the bacteriological findings were confirmed by an examination of duplicate samples of the affected lungs which were sent to Cambridge for the purpose.

Each of the outbreaks which occurred in other areas yielded an organism which proved to be identical with those obtained from North Wales and this seemed to be of interest in view of the fact that, as already explained, the circumstances under which they arose were somewhat different.

The number of organisms in the lungs varied considerably; in some instances they were comparatively scanty whilst in others the organ was extremely rich in these bacteria. The invasion of the pulmonary tissues seemed to be governed to a great extent by the severity of the lesions; thus in lungs which showed congestion without clear-cut consolidation, they were usually quite rare, whereas in those cases which had advanced to the stage of red or greyish-red hepatisation, the bacilli were extremely numerous. In general, the organisms were markedly pleomorphic. A smear from the cut surface of an affected lung revealed numerous cocco-bacilli, but all stages up to relatively long filamentous forms were also seen. In culture, the majority of the elements were in the form of short, slender Gram-negative rods from 1·5μ to 2·5μ in length and 0·6μ to 0·8μ in breadth, but longer filamentous forms, up to 5μ in length, were not uncommon. When stained by safranin or dilute fuchsin, there was little evidence of metachromatic granules in the organisms. The use of a blood stain, however, seemed to accentuate the polar type of reaction and a proportion of the smaller bacilli then assumed the appearance of typical pasteurella.

The organisms showed a distinct capsule both in the tissues and in cultures up to three to four days of age. The technique of Baker (1920) as used by Priestley (1936) in his study of *Pasteurella septica*, was employed and gave fairly satisfactory results. By this method, it was possible to demonstrate a clear halo around the organisms which was most pronounced in young cultures. All the strains which have been isolated so far have exhibited the formation of capsules, but unlike the true Pasteurella studied by Priestley, capsule formation was more marked when the organisms were grown on media containing blood or serum.

Cultural Characters.—Satisfactory cultures were obtained on plain, glucose or serum agar slopes. The colonies at 24 hours were

uniform, semi-translucent and of a whitish or greyish-white colour. They tended to increase in size with further incubation and might attain a diameter of 2 mm. to 3 mm. On serum agar, blood agar or chocolate agar, the colonies were larger, smooth with an entire edge. In broth, a uniform turbidity developed without pellicle formation. No evidence of motility could be detected in cultures grown at 22° C. and 37° C. respectively.

All the strains were slightly hæmolytic. The reaction was most apparent on sheep blood agar, but was also discernible on media prepared with blood from other species. On sheep blood agar, a well-marked clear zone of hæmolysis was observed at the 24th hour, particularly in the case of freshly isolated strains. With further incubation this area developed slowly reaching its maximum size at the 48th to 72nd hour.

The organism is relatively fragile when kept at room temperature or at cold room temperature on agar or in broth, but at 37° C. cultures retain their vitality for a longer period. Thus at room temperature it was frequently impossible to secure subcultures after three to four days even though the tubes had been stored in the dark, whereas the bacteria were still alive after 10 to 14 days at 37° C. Dungal (1931) has commented on the rapid death of the strains which he isolated from sheep and he states that at room temperature they were usually dead in from two to four days. In the early stages of the investigation, several strains were lost as the extreme delicacy of the organism towards laboratory temperatures was not fully appreciated. In order to avoid frequent subcultivation, we examined several types of media for the conservation of these organisms. The best results were obtained with cooked meat broth. The organism was grown in this medium at 37° C. for 18 hours and then stored in the cold room. Under these conditions we have found that our cultures have remained viable for at least eight weeks.

The fermentation of " sugars " was tested in peptone water containing 1 per cent. of the sugars. The reactions were fairly constant : thus acid without gas was produced almost with regularity in glucose, maltose, saccharose, lævulose, mannite and dextrin ; most strains fermented glycerine ; while about one-half fermented xylose. No change was observed with lactose, inulin, salicin, dulcite, arabinose and inosite. The following table gives the fermentation results with 24 strains.

	Positive.	*Negative.*		*Positive.*	*Negative.*
Lactose	0	24	Mannite	21	0*
Glucose	20	4	Dulcite	0	21*
Maltose	24	0	Inosite	5	19
Saccharose ...	24	0	Glycerine	15	8†
Lævulose ...	22	2	Dextrin	21	3
Arabinose ...	2	22	Inulin	0	24
Xylose	12	12	Salicin	0	24

*3 strains not tested. †1 strain not tested.

In the case of those sugars in which a comparatively high degree of acidity was produced, Andrade's indicator was satisfactory, but for the determination of a slight drop in pH value, more clear-cut results were obtained with brom-cresol-purple as an indicator (Lovell and Hughes, 1935). Litmus milk was unaffected and indol was not produced by any of the strains.

Pathogenicity.

In some of the natural outbreaks of sheep pneumonia a certain amount of presumptive evidence was obtained that the condition was contagious. In the majority of instances, however, it was difficult to obtain any direct information on the spread of the disease from infected to healthy sheep; moreover, isolated cases of the disease, the origin of which was entirely obscure, were not uncommon.

With the object of throwing additional light on the etiology of the disease, it seemed desirable to undertake a series of experiments in various animals, including sheep. The problem was attacked from two angles (*a*) a determination of the pathogenicity of the Pasteurella, isolated from the lungs of affected cases, for sheep and for smaller laboratory animals, and (*b*) attempts to set up pneumonia in sheep with unfiltered and filtered tissues from affected animals on the assumption that the disease might be caused by a filtrable or uncultivable agent alone or in conjunction with the organisms found in infected lungs.

The results of these experiments may be briefly described as follows.

(*a*) *Pathogenicity of the Pasteurella Strains.*—It was not possible to test every strain for pathogenicity but a proportion was selected for the inoculation of rabbits, guineapigs, mice and pigeons. In most cases, cultures which had been isolated directly from affected sheep, or had not been subcultivated for more than two or three generations, were used, but in a few instances well-established laboratory strains were similarly tested. It was found that the rabbit tolerated large doses (up to 2·0 c.c. of a 24-hour broth culture) by the intravenous, intraperitoneal and subcutaneous routes. Guineapigs and pigeons were also completely refractory to large doses given by various routes. Mice succumbed within 24 hours to three days when comparatively large doses (0·3 c.c. to 0·5 c.c. of a 24-hour broth culture) were injected intraperitoneally, but the strains soon lost their pathogenicity on subcultivation. The organism was without effect by other routes of infection. Attempts were made to augment the invasiveness of selected strains for the mouse by rapid serial intraperitoneal injections, but the minimal lethal dose was not appreciably augmented.

The low invasive powers of these strains, particularly in respect of the rabbit and guineapig, clearly differentiate them from *P. septica*.

The strains were also apathogenic for sheep when inoculated intravenously (two animals), subcutaneously (one animal) and when

instilled into the eye (one animal). The effect of the intranasal introduction of cultures will be considered under the next heading.

(*b*) *The Inoculation of Tissues from Affected Cases.*—(1) *Small Animals.*—Since the experiments undertaken with the organisms isolated from affected lungs had yielded negative results, attention was directed to the infectivity of the pulmonary tissues themselves. In some instances suspensions were tested which were prepared from comparatively early cases in which the lungs showed a stage of commencing congestion without consolidation, while in other instances areas of lung tissue showing well-marked red or grey hepatisation were used.

Portions of affected lungs were ground up with sand and a 5 per cent. suspension was made with broth. This was centrifuged at about 3,000 r.p.m. for ten minutes and the supernatant fluid used for the subcutaneous, intravenous or intraperitoneal inoculation of rabbits, guineapigs, ferrets and mice. The results were invariably negative.

Attempts were also made to infect small animals by intranasal instillation under ether and by direct inoculation into the lungs. In the case of the rabbit and the ferret it was impossible to provoke definite lesions. In the mouse, however, on two occasions a train of symptoms was set up that was not dissimilar from that which has been described in influenzal pneumonia in the mouse. In one instance twelve mice received each 0·05 c.c. intranasally of a suspension of consolidated lung from an affected sheep. On the fourth day, three of the animals were depressed and breathing rapidly. They were killed and at *post-mortem* examination each showed a distinct area of congestion involving one lobe of one lung. The remainder were killed at varying intervals but in no case were any distinctive pulmonary changes detected.

The lungs from the infected mice were triturated with broth, centrifuged and the supernatant fluid passed through a small Berkefeld V candle. This fluid was instilled intranasally under ether (0·05 c.c.) and injected directly into the lungs (0·1 c.c.) of twelve mice. On *post-mortem* examination two mice only showed definite lung lesions which were used to initiate a series of ten passage experiments in mice. After the third passage, the lesions in the lungs became more pronounced, the majority of the inoculated mice dying at the third to fifth day with pronounced areas of congestion and some consolidation of the lungs. Cultures from affected areas in the lungs were sterile. After the agent had been passed through ten generations, a suspicion was aroused that some of the animals of the batch then in use were infected with the virus of ectromelia. Further experiments in which a fresh stock of mice was inoculated intradermally in the foot with suspensions of infected mouse lung confirmed this fear, and no further passages were attempted. It is believed, however, that the lesions in the original series of mice were not produced by ectromelia virus, but were due to some other infective agent for those

mice were entirely free from macroscopic lesions of ectromelia. It is not possible, however, to exclude the supposition that this virus was present in a latent form and only made itself apparent when the animals were depressed by the intranasal or intrapulmonary instillation of the original or subsequent lung tissues.

This experiment was repeated with a lung suspension from a second case of ovine pneumonia using an entirely different stock of mice. An infective agent was again established by intranasal instillation under ether, but it proved impossible to infect mice beyond the fourth generation. These mouse strains were without effect on the ferret either by the intranasal route or by direct inoculation into the lungs.

(2) *Sheep.*—In addition to small laboratory animals, suspensions of infected lungs were also tested on sheep. The results of these experiments may be given in detail.

Experiment 472.—8.2.36. The material used was obtained from a portion of lung (Case 2, R.F.M.) showing a stage of advanced red hepatisation. A suspension was prepared in broth, lightly centrifuged and 0·5 c.c. of the supernatant fluid injected intratracheally into Ewe 60. The temperature of this animal remained normal and no clinical symptoms were observed. It was killed ten days later. About one-tenth of the lung tissue showed irregular patches of slight congestion which were most marked around the larger bronchi. No organisms were obtained in cultures prepared from these areas.

At the same time, Ewe 11 was lightly etherised and 15·0 c.c. of the same suspension was injected into each nostril. The animal remained quite normal for a period of three weeks when the experiment was discontinued.

Passage of portion of congested lung from Ewe 60 to Ewe 61 by intratracheal injection and by direct inoculation into the lung was without effect.

Experiment 830.—8.6.37. A lamb was received from a severe outbreak of ovine pneumonia in Norfolk. Extensive areas of consolidation were found in the lungs, from which a pure culture of the organism was isolated. A broth suspension of one of these lesions was lightly centrifuged and the supernatant fluid was used for the inoculation of three sheep.

Ewe 26 received 1·0 c.c. intravenously, 5·0 c.c. intratracheally and 0·5 c.c. directly into the lung. At the 24th hour, there was a marked respiratory distress while at the 48th hour the animal was very sick and unable to rise. It was killed. The *post-mortem* examination showed an extensive congestion of the anterior lobes of the lungs. On the right side there was a marked gelatinous deposit on the visceral pleura corresponding with the point of inoculation, while the underlying pulmonary tissues were congested. The pleural and peritoneal cavities contained a clear, serous fluid. An organism corresponding with that obtained from the original material was isolated in small numbers from one area of congested lung only.

Ewe 36 was injected in the same manner as *Ewe* 26. On the third day the animal was dull and showed increased respirations. These symptoms steadily increased in severity and the animal was found dead seven days after injection. The *post-mortem* findings were very similar to those observed in Ewe 26. The pasteurella-like organism was not isolated from the lungs, which contained *B. coli* in small numbers.

Lamb 20 was inoculated intravenously with 1·0 c.c. and intratracheally with 5·0 c.c. of the same suspension. On the fourth day, the lamb was depressed and affected with a husky cough. No further change occurred until the eleventh day when the animal began to lose condition. At the 16th day it was practically moribund and was killed.

At autopsy lesions similar in nature to those observed in Ewes 26 and 36 were found, but they were more extensive. The most marked feature was the extensive gelatinous exudate in the pleural cavity and a gelatinous œdema in the trabecular substance of the lungs. Cultures from the lungs, pleura, etc., were sterile.

Experiment 831.—28.6.37. Material from the lungs of Lamb 20 was emulsified in saline, centrifuged and the supernatant fluid injected into Ewe 21 by intravenous, intratracheal and intrapulmonary routes. The temperature of this animal rose to 106·2° F. at the fourth day and remained high until its death at the eleventh day. During this time the sheep did not exhibit any marked symptoms. The *post-mortem* examination showed that the lungs on the inoculated side (left) were adherent to the chest wall and the underlying pulmonary tissues were deeply congested and partly consolidated. The right lung showed marked congestion of about one-third to one-quarter its area. There was an extensive fibrinous gelatinous exudate in the pleural cavity. Cultures from the affected portion of lung was negative.

Ewe 73 was inoculated at the same time by the intravenous and intratracheal routes without result.

Experiment 850.—10.6.37. Ewe 75 was inoculated intravenously, intratracheally and directly into lung with affected pulmonary tissues from experimental Ewe 21. There was no result.

The results of these experiments suggest that although suspensions of congested or consolidated pulmonary tissues from natural cases of the disease are capable of inducing definite changes in the lungs of experimental sheep, it has so far proved impossible to establish an infective agent by direct serial passages. In regard to the routes of infection, intranasal instillation was without effect, while intratracheal inoculation only induced a mild form of localised pulmonary congestion. Much more severe results were obtained by combinations of intratracheal and intravenous, or intratracheal, intravenous and intrapulmonary injections. In these cases the experimental animals were rendered very ill and either died or were killed when *in extremis*. The pulmonary changes, however, were quite different from those which occur in the natural disease. The most striking feature was the production of an extensive fibrino-gelatinous exudate in the pleural cavity accompanied by a gelatinous œdema in the trabecular pulmonary substance. The microscopic appearance of sections of lungs from these experimental cases was also markedly dissimilar from that of natural material (see page 90). It was also remarkable that although the original lung suspensions used in these experiments contained large numbers of the Pasteurella organism, cultures from the congested or consolidated areas of the lungs as well as from the pleural œdema of the inoculated animals were, in most instances, remarkably free from this bacterium.

In addition to the attempts at inducing the disease by the inoculation of fresh tissues, the pathogenicity of the cultures isolated from the lungs was tested on sheep with the following results.

Experiment 480.—25.2.36. Twenty-four-hour broth cultures of two strains of pasteurella (third and fifth subcultures respectively) were mixed and 4·0 c.c. inoculated intratracheally into Ewe 34. The animal remained unaffected.

Experiment 490.—11.3.36. Twenty-four-hour broth cultures of the same two strains (fourth and sixth subcultures) were mixed. Lamb 53 received 3·0 c.c. intravenously and remained unaffected; 3·0 c.c. of the same broth culture was centrifuged and the organisms taken up in 0·2 c.c. of 3 per cent. agar which was run into a wide bore needle and allowed to set. The needle was then inserted into the jugular vein of Lamb 52 and the cylinder of agar forced into the blood stream by attaching a syringe charged with saline to the end of the needle. Daubney and Hudson (1934) have reported that they were successful in reproducing pleuro-pneumonia in cattle by a similar method and it was hoped that by the creation of an embolus in the pulmonary circulation these pasteurella organisms of the sheep might be enabled to establish themselves in the lungs. The animal, however, remained entirely unaffected.

Experiment 597.—8.7.37. Twenty-four-hour broth cultures of six pasteurella strains were mixed and inoculated into Ewe 37 which received 5·0 c.c. intranasally, 5·0 c.c. intratracheally and 1·0 c.c. directly into the lung. The animal remained healthy.

A second Ewe, No. 39, was inoculated subcutaneously with 5·0 c.c. of the same suspension, also without effect.

These experiments, therefore, afforded no evidence that the pasteurella-like organism itself was in any way pathogenic for sheep. It does not necessarily follow, however, that the bacterium plays no part in the causation of the natural disease. The observations of Shope (1931) and others on the etiological significance of mixtures of swine influenza virus and *Hæmophilus influenzæ suis* in the production of acute symptoms of swine influenza are particularly illuminating. The virus alone induces a reaction which is extremely mild; the organism alone is without effect, while a mixture of the two sets up a typical influenzal syndrome. It is conceivable that in the disease of sheep under discussion, the pasteurella has no primary etiological significance, but is enabled to establish itself through the agency of some other factor.

The attempt to determine the possible nature of such a factor if one were present was hampered by the failure to establish a transmissible disease in sheep by means of unfiltered suspension of lung tissue from natural cases. As, however, the experiments with mice seemed to be more successful it was decided to test the effect on sheep of a mixture of the agent transmitted to mice (which has already been described) and cultures of the pasteurella. In the first place it was found that a 5 per cent. suspension of normal mouse lung of itself was without effect. Ewe 38 received 5 c.c. intratracheally and 5 c.c. by intranasal insufflation under ether anæsthesia. Its temperature remained normal and no signs of pneumonia developed.

Two experiments were undertaken with the mixture, the details of which are as follows.

In each instance a 5 per cent. suspension of infective lung from several mice and a 24-hour broth culture of several strains of the organism were used. The animals were anæsthetised and 5·0 c.c. inoculated intratracheally while 5·0 c.c. was instilled into the nostrils.

Experiment 600.—8.7.36.

Sheep 50 received mouse lung only. It showed a slight rise in temperature from the 24th hour to the third day and then became normal.

Ewe 24 received culture only. It remained normal.

Ewe 23 received a mixture of culture and mouse lung. At the 24th hour it was extremely ill and was found dead 28 hours after inoculation. Both lungs were firmer than the normal. They showed extensive areas of congestion of both anterior lobes, particularly around the bifurcation of the bronchi and at the extremities of the two posterior lobes.

Experiment 616.—11.8.36.

Ewe 6 received mouse lung only. No rise in temperature occurred and the ewe remained healthy.

Ewe 31 received culture only. It also was unaffected.

Ewe 8 received a mixture of culture and lung tissue. There was a rise in temperature at the 24th hour which lasted for three days. During this period the ewe was somewhat depressed and its respirations were short and sharp. It made a complete recovery, however, and when killed its lungs appeared to be normal.

No precise conclusions can be drawn from these experiments. It has already been explained that the nature of the infective agent which was established in mice was not definitely ascertained. All that can be said at this stage is that suspensions of mouse lungs or cultures of the organism alone had no effect but that mixtures of these two injected intranasally and intratracheally, the combination produced a rise in temperature and in one case the animal died with signs of early pneumonia.

Agglutination Reactions.

A few of the strains were selected for the production of agglutinating sera. The organisms were grown on plain agar for 24 hours, washed off in saline and heated to 56° C. for half an hour. Rabbits were then given from six to eight doses intravenously at weekly intervals and were bled when their sera showed titres of from 1/640 to 1/2,560. The suspensions for the agglutination tests were prepared in a similar manner and were standardised to the opacity of a Brown No. 3 tube.

It was immediately found that the strains could be divided into two distinct sub-groups which will be referred to as I and II (Table I). Sub-group I contained 16 strains. The majority were isolated from cases of ovine pneumonia, but five calf strains also fell into this group. Of the latter, two were kindly sent to us by Dr. Lovell as representative of the pasteurella strain described by him (Lovell and Hughes, 1935). HP1 was obtained from a calf affected with pneumonia, while

N35 was isolated from the naso-pharynx of a normal, in-contact calf. Strains M, R5 and R6 were obtained by ourselves from outbreaks in adult cattle in which the symptoms were acute and death resulted from a broncho-pneumonia. Sub-group II contained ten strains including an ovine culture kindly sent from Iceland by Prof. Dungal.

Table I.

Antigen.	*Sub-group I Serum.*		*Sub-group II Serum.*	
	33	M.	2	D.
33	2,560	640	O	O
M	1,280	640	O	O
2	tr 40	O	320	640
D	O	tr 40	320	640
937	1,280	640	O	O
23	1,280	640	O	O
148	1,280	160	O	O
R1	2,460	—	tr 40	O
R2	—	640	O	O
HP1	1,280	—	—	O
R3	—	640	O	—
N35	1,280	—	O	—
65	1,280	—	O	—
857B	—	640	O	—
DN1	—	640	O	—
UF1	320	—	O	—
R5	640	—	O	—
R6	320	—	O	—
X	O	O	160	—
3	tr 40	tr 40	320	640
39	O	O	320	640
158	O	O	320	320
H1	O	—	320	320
SH1	—	O	—	320
SH2 Lung.	O	—	—	320
SH2 Trachea.	O	—	—	320
157	640	320	320	160
P. septica (avian)	O	O	O	O

— = not done. O = no agglutination 1/40.

As can be seen from the Table, sera prepared against two representative strains of sub-group I (M and 33) agglutinated all the strains of this group usually to titre and were practically without effect on sub-group II strains. The only exception was R1—a sheep strain—which gave a trace of agglutination (1/40) with one out of two sub-group II sera. No. 148 was also somewhat different since with serum 33 agglutination to titre was obtained whereas with serum M the end point in complete agglutination was reached at a dilution of 1/160. Sera against two representative members of sub-group II, one of which was the Dungal strain (D), behaved in a similar manner in that they agglutinated all members of their own sub-group. It was more difficult to secure good agglutinating sera in this group and

the titre was not so high as with sub-group I. The range of agglutination of all these strains with the exception of X fluctuated between 1/320 and 1/640. Nos. 2, 3 and D also showed a trace of agglutination at a dilution of 1/40 with sub-group I sera. It was noted that with sub-group I sera, agglutination was usually rapid and of a somewhat flocculent type, whereas with sub-group II visible clearing occurred slowly and was inclined to be more granular.

It will be seen from Table I that the only strain which up to the present has behaved in an aberrant fashion is 157. This organism appears to contain components belonging to each group since it was agglutinated to a titre of 640 and 320 respectively with sera 33 and M (I) and to 320 and 160 with sera 2 and D (II). At first it was thought that these results might be explained on the grounds that both types were present in the original lung tissue. This suggestion was found to be untenable, however, by a further study of the strain. The culture was plated twice, several single colonies being selected at random. After the second subcultivation, suspensions from 16 single colonies were tested against sera of the two groups: each behaved in a manner similar to the original culture.

The two sub-groups (I and II) were tested by agglutinin absorption to determine whether any further sub-division was possible. A sub-group I serum S 33 was completely absorbed by itself and by 23, but was not absorbed by a sub-group II strain 39. Conversely, a sub-group II serum 39 was completely absorbed by itself and by D, but was not affected by a sub-group I strain 33. (Tables II and III). A number of similar experiments with other sera and other members of the groups gave similar results.

Table II.

Antigen.	*S.33 Unabsorbed.*	*S.33 Absorbed with*		
		23 (*I*).	33 (*I*).	39 (*II*).
23 (I)	1,280	O	O	1,280
33 (I)	1,280	O	O	1,280
39 (II)	O	—	—	—

Table III.

Antigen.	*S.39 Unabsorbed.*	*S.39 Absorbed with*		
		39 (*II*).	*D* (*II*).	33 (*I*).
39 (II)	320	O	O	320
D (II)	640	O	O	640
33 (I)	O	—	—	—

Tables IV and V show that an absorbed serum of one sub-group did not agglutinate any other member of that group.

Table IV.

Antigen (I).	*S.*33 *Unabsorbed.*	*S.*33 *Absorbed with* 23 *(I).*
23	1,280	O
33	1,280	O
148	1,280	O
M	1,280	O
937	1,280	O
R1	2,460	O
R5	640	O
HP1	1,280	O
65	1,280	O

Table V.

Antigen (II).	*S.D. Unabsorbed.*	*S.D. Absorbed with* 39 *(II).*
D	640	O
2	640	O
3	640	O
39	640	O
158	320	O
41	320	O

Discussion.

Outbreaks of ovine pneumonia, both of a sporadic and an enzootic nature, have been recorded in the past, but up to the present the exact origin of such cases has not been satisfactorily explained. Dungal (1931) has recently given a very complete account of a disease which occurs in Iceland and which on epizootiological grounds, clinical manifestations and histological and bacteriological findings seems to agree quite closely with the condition which has been described in this paper. In every instance an organism of a pasteurella type was isolated from the lungs of affected sheep. Dungal has advanced the hypothesis that the primary cause of the Icelandic malady is this organism on the grounds that (*a*) it is found with constancy in the lungs of affected sheep; (*b*) that it is recoverable from the naso-pharynx of in-contact normal sheep in affected flocks; (*c*) that the intratracheal injection of cultures of this organism sets up in experimental sheep a syndrome indistinguishable from the natural disease. Leyshon (1932) gave a clinical description of an outbreak in sheep which was probably identical with those which we have investigated. A pasteurella organism was isolated, but it is not possible to say whether it was identical with the strains obtained in this investigation.

The isolation of pasteurella from the tissues of dead animals has often been advanced as proof that these organisms were directly responsible for the fatal issue, but there is substantial evidence to suggest that in many instances they are merely terminal invaders which have entered the organs in the last stages of the disease or after death. Sanders (1937), for example, has rightly pointed out that pasteurella have been recovered from the bodies of animals

which have died from a variety of causes, *e.g.* filterable virus diseases, plant poisoning and so on, and doubts whether they are of any etiological significance.

The question arises, therefore, as to whether this disease of sheep can be regarded as falling within the category of pasteurella infections. It may be said at once that our own investigations have not yielded a definite answer, but there are several points of interest which have emerged as a result of our enquiry.

In the first place what is the evidence that the malady is transmissible from affected to healthy sheep, and secondly what is the significance of the pasteurella isolated from lungs of diseased sheep ? One of us (R. F. M.) who has had an extensive experience of the disease in the field, has endeavoured to ascertain whether instances of direct transference from sheep to sheep were at all common. In some cases the evidence seemed to suggest that the introduction of an infected animal had initiated an outbreak : moreover, there can be no doubt that the crowding together of sheep often coincided with the appearance of pneumonia in a number of animals. On the other hand the rapid appearance in a flock of several cases of pneumonia was often associated with sudden changes in climatic conditions or the debilitating effects of prolonged and exhausting travelling. At one time it was thought that *Oestrus ovis* might be concerned in the disease since outbreaks often synchronised with the migration of the larvæ of this parasite, but as the investigation progressed it proved impossible to associate enzootic pneumonia definitely with this or indeed with any of the common parasites of the sheep. In East Anglia, for example, cases of the disease were encountered at widely varying times of the year and under diverse circumstances. In North Wales adult animals seemed to be highly susceptible while lambs usually escaped infection, but at least one outbreak was recognised in the Eastern counties in which a high incidence was encountered in young lambs. Taking the evidence as a whole, it has proved impossible to find any common environmental factor to account for the initiation of outbreaks under widely diverse conditions.

In nearly all cases it was possible to isolate a pasteurella-like organism from the lungs of affected animals. The strains thus obtained were compared with a culture kindly sent by Prof. Dungal with which, on broad grounds, they were in complete agreement. It is necessary to point out, however, that these organisms can only be recovered with certainty from animals which have succumbed to the condition and in which the pulmonary changes are advanced. In the early stages of the disease they are very scanty and may even be entirely absent. In our opinion this is a circumstance which places the entity outside the group of " hæmorrhagic septicæmias " and it may cast doubt on the primary etiological significance of this organism, although there is even stronger evidence, which will be discussed later, which supports the latter contention.

It is not proposed in this paper to deal at length with the classification of Pasteurella, but a brief reference must be made to the various sub-divisions of this group which have been suggested. Cornelius (1929) made a serological study of 26 strains of pasteurella obtained from a variety of animals. He used agglutinin absorption and complement fixation tests and divided his strains into four sub-groups and one which defied identification. Yusef (1935) experimented with the precipitin test and detected differences in antigenic structure between certain pasteurella strains by a precipitin absorption method. The results were fairly consistent but were not, on the whole, much better than those obtained by agglutination tests. Brigham and Rettger (1935) on the other hand, who examined 78 strains, came to the conclusion that the results of agglutination tests were disappointing and this has been the experience of other workers. In 1921, Jones and Little isolated several types of pasteurella from calves affected with pneumonia, some of which differed from the classical organisms. They divided their strains into three groups termed I, II and III respectively. Groups I and II were encapsulated and were of low virulence for rabbits and mice. Group III which was pathogenic for rabbits, was regarded as belonging to the hæmorrhagic septicæmia class. Indol-positive pasteurella have been isolated from sheep but the relative infrequency of references in the literature to this organism as a cause of ovine pneumonia would seem to imply that such cases are comparatively rare (Newsom and Cross, 1923; Henning and Brown, 1936; Fitch and Nelson, 1923). The bacteria isolated from sheep in Iceland and in this country, however, have a marked resemblance to Jones' group I. Both organisms are encapsulated, hæmolytic on blood agar, ferment glucose, saccharose, maltose and mannite, and are indol negative. There is a difference in the action on lactose since Jones' strains fermented lactose slowly whereas the sheep strains failed to affect this sugar even after prolonged incubation. It is possible, however, that true representatives of the Jones group I do occur in sheep since Newsom and Cross (1923) isolated from cases of ovine pneumonia both Jones' group III and also organisms which they regarded as pertaining to group I. It may be noted in passing that Tweed and Edington (1930) and Lovell and Hughes (1935) have reported slow lactose fermentation in strains of a similar nature which they have isolated from bovine animals whereas four calf strains which we have obtained from lungs showing acute pneumonia, have behaved as the ovine organisms.

On serological grounds the sheep strains are sharply differentiated from the recognised members of the Pasteurella group with which they have been compared. They are, however, related to the strains isolated by us from cases of pneumonia in cattle which together with two of bovine origin obtained from Dr. Lovell fell into our sub-group I (Table VI).

Table VI.

Organism.	*Serum P. avisepticus.*	*Serum Dungal.*	*Serum Sheep Sub-group I.*	*Serum Sheep Sub-group II.*
B. pestis	—	O	O	O
P. pseudotuberculosis ...	—	O	O	O
P. Jones Group I... ...	—	O	O	O
P. „ „ II ...	—	O	O	O
P. „ „ III ...	—	O	O	O
P. avisepticus	160	O	O	O
P. bovisepticus	160	O	O	O
Dungal	O	640	O	640
Sheep (Sub-group I) ...	O	O	1,280	O
Sheep (Sub-group II) ...	O	640	O	640
HP1 (Calf. Lovell) ...	—	O	1,280	O
M (Calf. I.A.P.)... ...	—	O	1,280	O

— = not done. O = no agglutination 1/40.

This Table clearly shows that there is no serological relationship between *P. pestis*, *P. pseudo-tuberculosis*, *P. jones* groups I, II, II respectively, *P. avisepticus*, *P. bovisepticus* and the ovine strains. As indicated in the paper, the last named can be divided into two groups (sub-groups 1 and 2) which are antigenically different, Dungal's strain falling within one of these sub-groups.

It has already been pointed out that there are valid reasons for doubting whether these pasteurella organisms are solely responsible for the disease we have described. Jones and Little remarked on the low virulence of their group I organisms and reported that 2·5 c.c. of pleural fluid from an affected case inoculated subcutaneously into a calf was without effect, while the intratracheal injection of 10 c.c. of a 24-hour broth culture into a second calf also failed to produce any symptoms. Sanders attempted to infect calves by spraying them with cultures of *Past. boviseptica* obtained from clinical cases of hæmorrhagic septicæmia, but apart from a slight catarrh, no visible ill effects were noticed and the organisms were not recovered from the blood stream. In Dungal's experiments, on the other hand, sheep were inoculated intratracheally with broth cultures of the organism which he isolated from affected animals, and in some instances succumbed later with pulmonary lesions resembling those of the natural disease. Newsom and Cross also state that the intravenous injection of 10 c.c. of broth culture of a pasteurella organism identified as *P. ovisepticus* resulted in death in 24 hours.

In our own experiments several attempts were made to induce a pneumonic condition in sheep by the inoculation, by various routes, of cultures of the pasteurella organism isolated from the lungs, but the results were quite negative and were thus contrary to those reported by Dungal. As the organisms used were freshly isolated the criticism cannot be levelled that they were lacking in virulence through prolonged cultivation in artificial media.

On the other hand direct inoculation of suspensions of unfiltered affected lungs from acute cases of the condition induced the formation

of lesions without, however, exactly reproducing the natural disease. Unfortunately, we were unsuccessful in securing serial passages in other sheep and also failed to find a small animal which was susceptible, with the possible exception of the mouse, so that no precise deductions can be drawn from our experiments. It is true that there was some indication that a transmissible filterable disease could be induced in mice by the intranasal instillation of infective material, but inasmuch as the mice were not entirely free from suspicion of a concurrent infection with ectromelia virus, we feel that the evidence on this point may be untrustworthy.

Nevertheless, the impression has been gained as a result of this investigation that, whilst the pasteurella may be largely responsible for the severe terminal pneumonia, it is highly probable that it is unable to gain an entrance until a breach has been effected by some other cause. It is known that these organisms inhabit the upper respiratory tract of sheep and cattle (Dungal, Jones and Little, Lovell, etc.) but their invasive powers are probably low. Whether the initial stimulus is due to the action of a filterable virus or is dependent upon some environmental change has yet to be determined.

Summary.

1. A description is given of an enzootic form of pneumonia in sheep, outbreaks of which have been investigated in North Wales and East Anglia. It bears a close resemblance to the condition described by Dungal in Iceland.

2. An organism nearly related to the Pasteurella group has been isolated from affected lungs showing an advanced stage of hepatisation. The strains thus obtained closely resemble, on cultural and biochemical grounds, group I pasteurella (Jones and Little), *i.e.*, they are encapsulated, haemolytic, indol negative and of low pathogenicity for experimental animals. Serologically, however, they appear to fall into a group of their own since sera prepared against selected cultures failed to agglutinate any of the recognised members of the pasteurella group.

3. Agglutination tests with sera prepared against a number of the sheep strains led to their division into two distinct sub-groups termed I and II. Sub-group I sera agglutinated all the members within the group to about the same titre but failed to agglutinate sub-group II, and *vice versa*. A strain representative of the disease of Icelandic sheep which was obtained from Prof. Dungal appeared to be identical with our sub-group II. Certain strains isolated from cases of pneumonia in cattle in this country fell into sub-group I.

4. Organisms cultured from affected animals were tested on experimental sheep by various routes but even when freshly isolated they appeared to be devoid of pathogenicity. On the other hand, severe pulmonary changes were induced by the intravenous and intrapulmonary inoculation of suspensions from affected lungs. The

changes were not entirely typical of those encountered in the natural disease.

5. No direct evidence was obtained as to the transmission from diseased to healthy animals in affected flocks. It is suggested that outbreaks may be precipitated by sudden environmental changes and may even be associated with a sharp change in weather conditions.

We wish to acknowledge the valued co-operation of Mr. W. T. Rowlands, of Bangor, in the collection and examination of material; also to thank Professor N. Dungal, of Iceland, and Dr. R. Lovell, of London, for kindly supplying us with cultures.

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EQUINE CONTAGIOUS PNEUMONIA.
GERMAN *BRUSTSEUCHE.*

By Sir John M'Fadyean.

Aetiology.

The disease has two causes, which act separately and are responsible for different stages in the illness and different lesions. One of these is a virus, and the other is a visible organism—a streptococcus.

The virus has not been made visible or cultivated outside the body. It is the veritable cause in the sense that it is exclusively responsible for the spread of the disease and also for the primary lesions, which are in the bronchi and not in the parenchyma of the lungs.

The streptococcus is responsible for the pneumonia which gives the disease its serious character and in fatal cases is generally the actual cause of death. It is indistinguishable from streptococci that are commonly present in small numbers in the air passages of normal horses. It is readily cultivated and recognisable by the microscope.

The evidence in proof of these statements will now be stated.

Schütz, working in Berlin, in 1886, set himself to determine the cause of *Brustseuche*, and the results of his researches were published in 1887.

He found that the solid parts of the inflamed lungs in cases of the disease constantly contained in great abundance an organism which he termed a diplo-streptococcus. He cultivated this organism in series in peptone beef infusion, and with part of a culture of the twelfth generation he inoculated a mouse. It died on the following day, and its blood, which contained the organism in question in a state of purity, was used to inseminate another flask. Part of this last flask after cultivation was used for the inoculation of a horse, and in a second experiment a yearling colt was made to inhale liquid culture of the organism in the form of a spray.

At the outset of his investigations regarding *Brustseuche*, Schütz decided to follow the plan prescribed by Koch for research in contagious diseases (so-called Koch's postulates), which he stated as follows :—

(1) In the diseased organs of the animal the same organism must always be demonstrable.

(2) This organism must be isolated and cultivated from tube to tube until its absolute purity has been determined.

(3) Lastly, the organism must be transmitted to animals of the same species or to susceptible animals of another species. When this determines the development of a disease identical with the one from which the pure culture of the organism was obtained the proof has been brought that the latter is the cause of the disease.

In summing up the result of his researches Schütz claimed :—

(1) That in the lungs of horses affected with *Brustseuche* he had found, in large numbers, a particular organism,

(2) That he had been able to isolate this organism and study its mode of growth.

(3) That inoculation of the organism into the lungs of sound horses caused a disease identical with the one from which he had obtained the organism, *Brustseuche*.

The following notes refer to the two animals which were inoculated with the culture of the streptococcus.

On the 30th of June, blood of a mouse, which had been inoculated with the twelfth generation of the bacteria and died on the following day, was used for inoculating peptone beef infusion, which was cultivated at 35° C. till the 2nd of July. It then had a thick white flocculent mass at the bottom of the flask, and, on examination, it was found to be exclusively made up of the streptococcus.

From this culture on the 2nd of July a two-year-old horse was inoculated with a Pravaz syringe by injecting 4 c.c. into the lungs at four different places, two on the right side and two on the left. The author says, " It was my intention by the inoculation of pure cultures to produce necrotic centres in the lungs, similar to those found in gangrenous pneumonia ; and in order to favour the development of a perforation in the pleural sac the cannula was introduced only as far as the outer layer of the lung, in order that the centres would develop in as superficial a position as possible." Simultaneously a mouse was inoculated subcutaneously on the back with two drops of the same culture. (The mouse died two days later and the *post-mortem* showed the usual lesions). The following notes refer to the horse.

On the 3rd of July (the day after the inoculation) food had not been touched by the horse, which was frightened by noise and shivering. Pulse small and irregular, conjunctiva reddened, nasal mucous membrane highly reddened, mucous membrane of the mouth rather dry ; increased thirst ; breathing frequent ; pain at the points of inoculation. Weak respiratory sounds on auscultation of the left side, and weak friction sounds over the places where the punctures had been made. Breath sounds nearly suppressed on the right side over the inoculation site.

4th July. Rather brighter, appetite very slight, considerable thirst ; some turbid mucus from the nostrils ; from time to time short cough ; breathing superficial, very rapid ; groaning on pressure of the chest wall.

5th July. Still brighter. Appetite better ; thirst slight. Pressure on the chest wall painful ; breathing mainly abdominal.

6th July. Discharge from nose ceased. Mucous membrane dry. Artificially produced coughing very painful, short and without strength ; respiration purely abdominal ; friction sounds on auscultation ; groaning on pressure over the chest wall.

The foregoing does not include all the facts recorded regarding the symptoms and the notes for the remaining days are omitted. The horse died on the evening of the 11th of July, and the *post-mortem* was made on the following day.

In the left pleural sac there were 7 litres and in the right 12 litres of a dirty yellow turbid liquid with coarse grey flocculi in it. The right lung was firmly attached to the chest wall at two places and there were also two points of attachment by growth of the left lung to the chest wall. Everywhere else the pleura was covered with a soft, firmly adherent, dirty yellow layer, which was most abundant around the right anterior attachment. The lung

had retracted from the chest wall. On cutting into the lungs it was found that the tissue at the right anterior adhesion was penetrated by a fine canal which opened with a round opening in the right pleural sac, and inwardly into a cavity as large as a hen's egg, which was situated near the surface of the lung. The cavity was filled with a soft, yellow, necrotic mass of lung, and its wall was irregular and greasy.

At one part in the right lung there was a large area of firm lung tissue which on section had a dry, yellow, close appearance. The lung around this was firm, airless and dark red. In the neighbourhood of the left adhesion there was a cavity as large as a hen's egg which contained dry yellow contents, easily washed out, leaving rough walls.

" The experiment had thus given the desired result, since after inoculation of pure culture of the bacteria into the lungs there developed at the sites of the inoculations a gangrenous inflammation of the lung which differed in no way from that of *Brustseuche*.

" At each of the places of inoculation there was what corresponded to a pneumonia with a special developmental history. The centre of the pneumonic area was made up of inflamed dead lung tissue, and the periphery of freshly inflamed lung tissue. There had thus been experimentally produced a multiple gangrenous pneumonia which was the product of the injected pure culture of the *Brustseuche* bacteria. The dead material had the same appearance as in *Brustseuche*, and showed the same sharp boundary, and further outwards there was the same fresh irritation zone. At three inoculation places there was softening of the dead mass and cavity formation, which, in the author's opinion, must also be ascribed to the action of the bacteria, and one cavity had caused the perforation into the left pleural sac and the fatal pleurisy. The disease had thus run exactly the same course as it does under natural conditions."

All the exudate masses in the thoracic cavity, and the dead and freshly inflamed parts, and all the organs of the horse when examined microscopically were found to contain the *Brustseuche* bacteria.

Material from this case was used for the inoculation of mice, with the usual results.

A colt about one year old, and completely sound, served for the following inhalation experiment.

On the 10th of August the trachea was opened and a tracheotomy tube was inserted, and two days later there was passed through it the sterilised tube of a spray apparatus, 18 cm. long and bent downwards to reach near the point where the trachea divides, and with this meat infusion culture of the *Brustseuche* bacteria was sprayed. For filling the apparatus seven Ehrlenmeyer flasks of culture were required. About 3 o'clock in the afternoon one half, and two hours later the other half, of the liquid was sprayed, half an hour being required for each operation. A short time after the end of the experiment there was hurried respiration and rattling sounds in the trachea and its large branches, and coughing from time to time, with the discharge of coarsely foamy liquid out of the tracheotomy tube. The pulse rate rose to 60 and the temperature to 39·2° C.

On the next day there was dullness in the under part of the right lung. The temperature fell quickly and the respiratory movements also. The dullness in the lower part of the right lung continued.

The colt was killed on the 5th of September and an accurate examination of the lungs was made. It was found that the under part of the right lung

was in a condition of chronic inflammation. The tissue was firm, airless, greyish-red and beset with small white specks, which without any sharp limit merged into the surrounding air-containing tissue. The wall of the smaller bronchi was filled with mucus.

The author remarked that chronic pneumonias in the horse very frequently exhibit at the outset the same picture as in the foregoing experimental colt. The position and shape of the indurated parts showed that the chronic inflammatory process had begun in the alveolar tissue of the lung and had led to an acute pneumonia. He had often observed that the acute fibrinous pneumonia of the horse when it did not lead to resolution led to chronic alterations in the lungs. It was therefore to be assumed that in the present experimental horse the chronic conditions in the lungs were the result of an acute inflammation which had been caused by the inhalation. The clinical symptoms also supported that view.

Schütz, however, decided that there must be another experiment of the same sort, but he thought he was already justified in concluding that in the reported experiments only the oval bacteria, freed from all the original morbid products, which he had isolated from the lungs of horses dead from *Brustseuche*, were the cause of the disease—in other words, that *Brustseuche* was an infectious disease caused by these bacteria. He carried out a third experiment, in which liquid culture of the diplococcus was injected into the lung of a six-months-old foal, which at the time had not completely recovered from a previous inoculation with strangles.

The experiments on which Schütz relied to prove that he had discovered the cause of *Brustseuche* did not strictly conform to " Koch's postulates." The last of these required evidence that the alleged culture of the causative organism had reproduced " the disease." In the present case the last two words of course meant *Brustseuche*. Schutz, however, produced no evidence that the disease set up by the streptococcus culture was contagious, and an infection which is not transmissible by contact is not *Brustseuche*. Nevertheless in the following years it was very generally accepted that the ætiology of *Brustseuche* had been made plain. In Germany numerous attempts were made to reap the advantage of this discovery by using cultures of the diplococcus either as a vaccine or to obtain specific immune serum. Experiments embracing thousands of army horses were carried out, but many of them were foredoomed to failure because they included no controls or only an inadequate number. Eventually it was realised that *Brustseuche* could not be prevented in this way, and as the matter was a very serious one, especially for the Army, Robert Koch, the world-famed bacteriologist, was commissioned to institute fresh investigations.

These were begun and carried on with the assistance of Army Veterinary Officers and others, and the first conclusion drawn by Koch was that the streptococcus was indistinguishable from organisms of the same class commonly found in the air passages of normal horses, and therefore could not be the cause of *Brustseuche*.

The Virus.

After Koch's death in 1910 Gaffky and Lührs continued the search for the cause of the disease, but for a long time without success, although further evidence was provided that Schütz's diplococcus was not the cause. During this period the study of the disease was made on experimental horses that had been exposed to infection by contact, isolated afterwards, and kept under close observation. They were killed for *post-mortem* examination when infection was manifested by the onset of symptoms of pneumonia. All the attempts to transmit the disease by inoculation with blood, diseased lung tissue, or material from other organs of these horses failed.

In retrospect it is obvious that they were bound to fail, because, as the later experiments proved, the virus, which alone can transmit the disease, disappears from the body before the stage of pneumonia is reached. The discovery of the virus afterwards was mainly due to an important change in the plan of the experiments. The horses experimentally infected by contact were killed as soon as they exhibited signs of illness, instead of waiting till the stage of actual pneumonia had been reached. In such cases *post-mortem* examination showed no gross lesions in the parenchyma of the lung, but only bronchitis with a clear gelatinous exudate in the smaller tubes.

As far as could be ascertained by microscopic and cultural examination the exudate in the small bronchi contained no visible bacteria, but it was proved to be capable of infecting horses when rubbed into the mucous membrane of the nostrils or mouth. Animals thus infected developed the disease as in natural cases, and they were capable of transferring the disease to other horses by contact.

In the experimentally infected animals killed three or four days after the onset of the visible illness bronchitis with the gelatinous exudate in the tubes was again found. The experiments had thus proved that the primary cause of *Brustseuche* is a virus.

The later investigations were facilitated and made less expensive by using foals or yearlings in the experiments, and as it was essential that the animals selected for experiment should be free from any suspicion of previous infection with *Brustseuche*, they were obtained from breeding studs in which evidence of such freedom extending backwards over a period of years could be obtained.

The following facts regarding the virus have an important bearing on the course of the disease :—

It has been found only in the lungs, and there only in the first stage of the disease, *i.e.*, the primary fever, which lasts about three or four days.

The blood has not been found infective at any stage of the disease.

The virus disappears from the lungs *pari passu* the development of the pneumonia.

The viability of the virus outside the body appears to be feeble.

It is pathogenic only for the horse and ass.

Attempts to transmit the disease by subcutaneous or intravenous inoculation of virus-containing material have failed.

Course of the Disease.

The earlier experiments carried out by the German Committee appeared to show that the period of incubation varied greatly in length in cases infected by contact, but in these the exact hour or day on which infection actually occurred could not be determined, and since the end of the period was dated from the onset of symptoms of pneumonia the calculated length was too long.

It was, however, found possible to make accurate measurements of its length in the later experiments, in which animals were infected by rubbing material containing the virus into the mucous membrane of the nostrils or mouth, and temperatures were recorded twice daily afterwards in order to detect the primary fever which marks the true end of the incubation period. In three foals infected in this way simultaneously the respective periods were 19, 18 and 39 days.

The duration of the primary fever during which the virus is propagating in the bronchi apparently varies little, and is usually three or four days. Formerly, in outbreaks occurring naturally, when animals were submitted only to clinical inspection, the primary stage of the disease was generally overlooked.

Apparently both in natural and experimental cases the disease may terminate with the return of the temperature to the normal *without the development of pneumonia*. This serves to explain observed cases in which horses involved in an outbreak appear to escape infection but are later found to be immune.

In consequence of the unusually long period of incubation, in a large stud of horses the outbreak may develop slowly and in an erratic manner, the disease apparently jumping from one part of the stable to another.

This is in contrast with what is observed in outbreaks of equine influenza, in which the horse nearest to one that has developed the disease is most likely to be the next attacked.

In what may be called an average case of the disease a rise of temperature is the earliest evidence of infection ; within two or three days it may rise from normal to 41° C. or more, and then fall in two or three days to normal and remain there with only slight fluctuations.

The respiratory disturbance of course varies with the progress of the disease in the chest, that is, the extent of the croupous consolidation of the lung tissue and of the collapse caused by pleuritic effusion in some cases. In fatal cases the respiration may rise to 40 or more per minute and the animal then exhibits great distress.

It might be thought that in view of the discovery of the invisible virus as the actual agent of infection, it was no longer necessary to take any account of the streptococcus in connection with the disease. That, however, is not the case, as the following considerations will show.

The researches of Gaffky and Lührs revealed the fact that in order to obtain this virus for experiment the foals or colts had to be killed at the end of what may be called the first stage in the disease, that is, about the third or fourth day after the first signs of illness, and before the development of the pneumonia. It thus appears that while there is adequate evidence to prove that the invisible virus is responsible for the onset of the disease there is equally good evidence that it disappears within three or four days, and therefore cannot be held responsible for the pneumonia, which, as already said, is the cause of the severe illness and of death in the fatal cases.

The Streptococcus.

The facts recently discovered concerning the role of the virus thus leave the occurrence of the pneumonia, which gives the disease its name, unexplained. They are, however, in no way inconsistent with the view that the streptococcus is responsible for the pneumonia. In this connection it must be remembered that the streptococcus is here not an inoffensive organism propagating in dead tissue. It belongs to the same group as the common pyogenic streptococci, and, as Schütz proved, its cultures are fatal by inoculation to mice and seriously pathogenic for the horse. Furthermore, in the inflamed lung the streptococcus is not confined to the dead tissue; on the contrary, it is found in the pneumonic lesions at all their stages, in numbers proportional to the severity of the structural alterations, and unmixed with other organisms or greatly predominant in number. It is only in the last stage of the disease that it may become outnumbered by bacteria of a putrefactive type, and even when death occurs a pure culture of the streptococcus can generally be obtained from the solid lung.

Lesions.

In the great majority of cases, including all those that terminate fatally, the *post-mortem* examination shows pneumonia, generally very extensive and accompanied by pleurisy. The pneumonia and pleurisy may be unilateral or bilateral.

The pneumonia usually begins at multiple centres throughout the lungs, but these soon become merged to form large masses. Microscopic examination of these areas show the picture of a bronchopneumonia, the air cells and minute bronchi being filled with an exudate in which the cells are mainly polynuclear leucocytes. As already stated, Gaffky and Lührs were able to examine early lesions in which there were no demonstrable bacteria, but as a rule lesions that are small but macroscopic already contain collections of diplococci or streptococci, apparently in a state of purity. Around these centres there is marked congestion of the lung tissue, and by extension of such primarily independent centres large areas of the lung become solid with the firmness but not the histology of the early stage of what is commonly called croupous pneumonia. Before death more

than half of one lung may be in this solid condition, but when pleurisy is added that is usually the immediate cause of death.

Contrary to what has often been stated, this pneumonic consolidation has little in common with the stage of red hepatisation in acute pneumonia of the human subject; essential differences are that the consolidation has been brought about by the extension of what were originally independent foci, and the histological picture is not uniform over large areas. Areas showing a uniform picture of a fibrinous coagulum are conspicuous by their absence in equine pneumonia. A striking and characteristic feature in connection with the lesions is their tendency to early necrosis. At a certain stage in the disease the solid lung is beset with white homogeneous-looking areas, which have often been erroneously described as abscesses. Between these areas the solid lung is intensely congested and generally shows hæmorrhages at some places. The process of necrosis extends and the dead tissue undergoes complete softening and becomes of a dirty greyish tint. When a lung at this stage is incised one can, under a tap, wash out completely this softened necrotic lung tissue and leave irregular cavities sometimes as large as one's fist. By the time that the process of softening has begun the lesions have a putrid odour, and microscopic examination then always shows a mixture of bacteria, which may now not include streptococci. This is the common condition of the lung in cases terminating fatally about a week after the onset of the first symptoms.

The pleurisy is generally secondary to the disease in the lung tissue and the pleural cavity contains a large amount of exudate, with more or less fibrinous lymph deposited on the membrane. When the before-described necrotic and gangrenous lung lesions are present the pleurisy is generally also of a putrid character and contains streptococci and other organisms.

The naked-eye appearance of the lesions at the different stages in equine pneumonia have long been well known and are, of course, essential for the diagnosis of the disease *post-mortem*. The microscopic structure of the lesions is no less important, but hitherto has not been adequately studied.

As opportunities were presented the author secured lesions from a number of representative cases of the disease, which after fixing, preserving and staining provided material for microscopic examination, and for taking micro-photographs, without which accurate description would scarcely be possible.

The lesions which are here figured were found in three different cases of equine pneumonia which died in the Infirmary at the Royal Veterinary College, London, and were post-mortemed by the present author.

The first was a chestnut mare (No. 2382) that died on July 9th, 1903, from what was diagnosed as "gangrenous pneumonia."

The second animal was a gelding (No. 2659) that died on June 30th, 1906. The solid patches of pneumonia had the appearance of early

necrosis and microscopic examination of smears from these patches showed immense numbers of diplococci in the grey areas.

The third animal, a gelding (No. 3491) died on May 3rd, 1916, from equine pneumonia after having been only two days in the College Infirmary. The *post-mortem* examination was made two hours after death and selected pieces of the solid lung were immediately fixed in formalin in normal saline. These included lesions of visible early necrosis, which were slightly putrid in odour and in which breaking down of the tissue had begun. Other parts were preserved from early lesions in which hepatisation was not complete. At some places there was thickening of the pleura over lung that had collapsed, but there was no fibrin on the surface and only a small amount of liquid in the chest cavity.

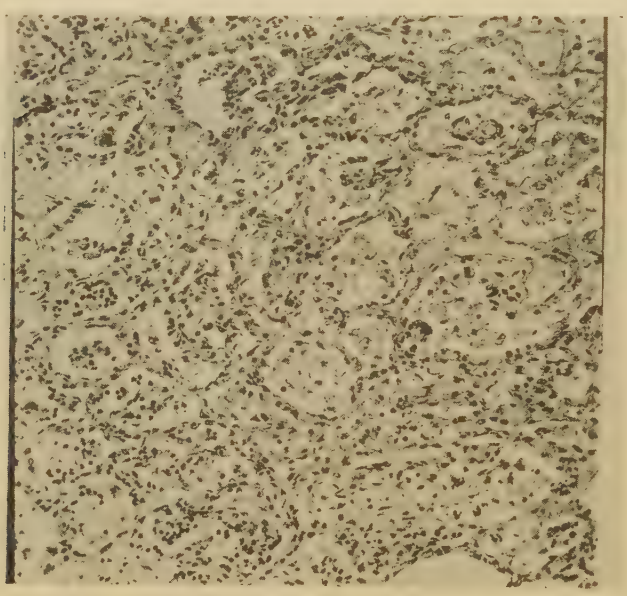

Fig. 1

There was extensive consolidation of both lungs, but more particularly of the right, in which the only unaffected part was a narrow strip about two or three inches wide running along the upper margin. The rest of the lung was quite solid and incision into it showed that it was beginning to become gangrenous.

The numbers prefixed to the different photographs indicate the horses from which they were obtained, the number of the microscopic section, and the magnification.

Fig. 1. 24/8. Equine Pneumonia. From 2382. × 134. The alveoli are occupied by epithelium which has been shed and is degenerating. No large collections of diplococci were found in these

alveoli or in any part of the section. This may have been the result of toxin diffusing in the part.

Fig. 2. 21/36. Equine Pneumonia. From 3491. 12 mm. Searcher oc., Cam. 30. × not recorded. The dark body near the centre is a clump of diplococci. In a Gram-W. parallel preparation of 3491 diplococci were found only in alveoli with cellular contents, scattered in those with little or no degeneration, and massed where necrosed. None in the alveoli with croupous exudate.

Fig. 3. 23/8. Equine Pneumonia. From 2659. × 102. Under a higher magnification it was seen that nearly all the cells in the alveoli were polynuclear leucocytes, but a few were apparently degenerated shed epithelial cells. This was the predominant form of exudate in the section, but at some places groups of alveoli contained red corpuscles and others homogeneous or fibrinous exudate with few cells. All the bronchi contained polynuclear exudate and epithelium that appeared dislocated or was actually detached. This applied to the ultimate bronchi as well as to those with several strata of epithelium.

Fig. 4. 12/44a. Equine Pneumonia. Early stage. From 2659. The cloudy part near the centre is necrotic nuclear contents in which numerous streptococci (stained with hæmatin) were embedded. Observe the thinness of the alveolar walls, which appear to have lost their epithelium. This was well seen in other fields in the same section showing naked capillaries still containing well preserved (circulating) red cells in them. The gradual destruction of capillary walls in this way appears to be one cause of infiltration of the alveoli with blood (the other cause being simple rupture of greatly distended vessels). All the recognisable cells in the alveoli are polynuclear showing commencing fragmentation of the nuclei. Part of another alveolus at the bottom right-hand corner also shows a smaller patch, dark stained, which is also composed of necrosed exudate containing streptococci.

Fig. 5. 28/22. From 3491. × 98. It shows a group of alveoli filled with exudate which is crowded with masses of diplococci. At the upper and lower edge of the section the alveoli are mainly occupied by blood.

Fig. 6. 28/21. Equine Pneumonia. From 3491. × 100. Shows a divided bronchus filled with exudate, in part distinctly cellular and partly necrosed, the latter containing masses of diplococci. The material in the bronchus must have been alveolar in origin. It is partly composed of polynuclear leucocytes but it also contains shed epithelial cells. It will be observed that the bronchial epithelium is still intact. Some of the air vesicles are empty and distended and the others contain a homogeneous exudate without fibrin.

FIG. 7. 32/18. Equine Pneumonia. From 3491. No. 3 Reich., No oc., Cam. 36. Stained Gram. The dark stained material is entirely composed of collections of diplococci stained by the gentian-violet, in lung tissue undergoing necrosis.

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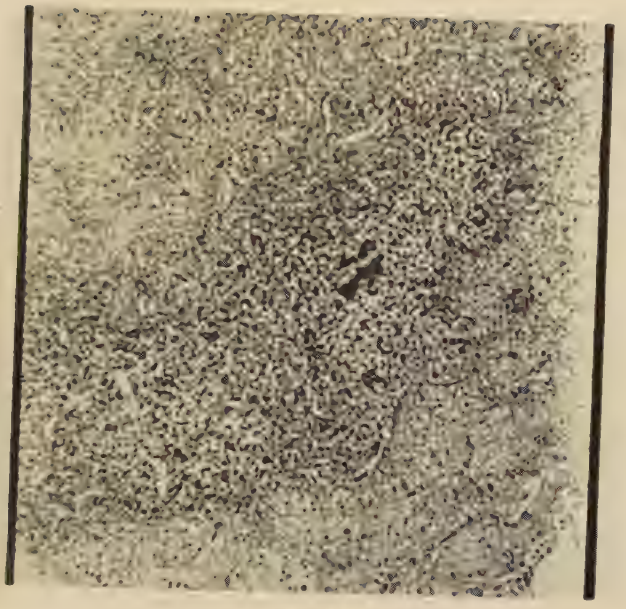

Fig. 2

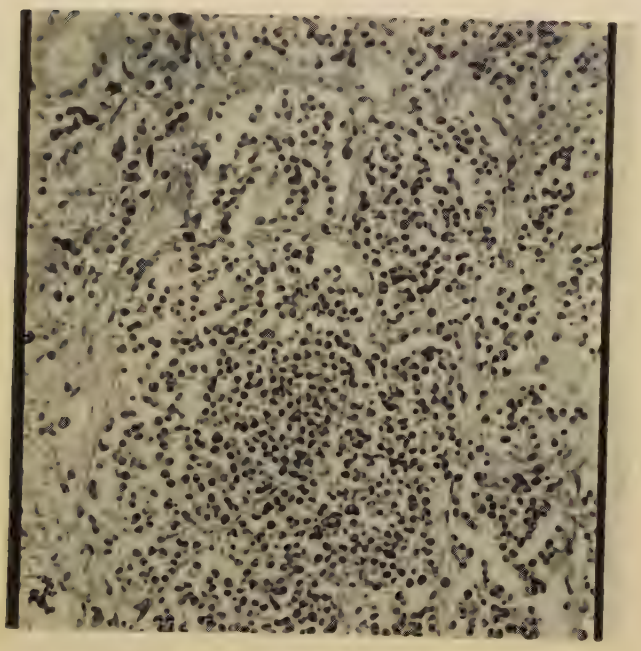

Fig. 3

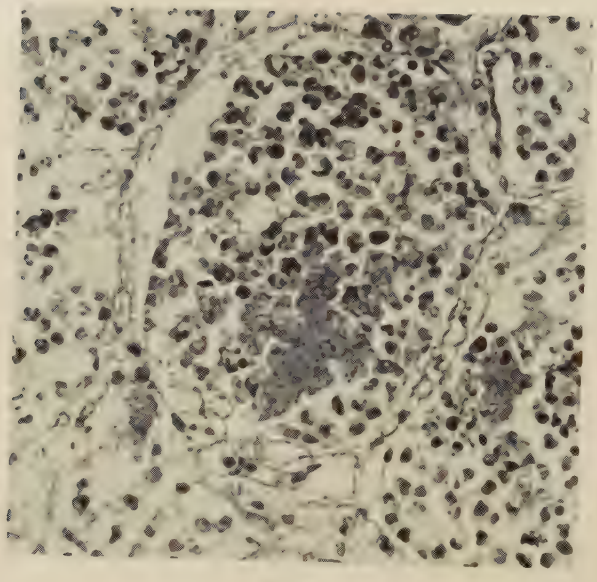

Fig. 4

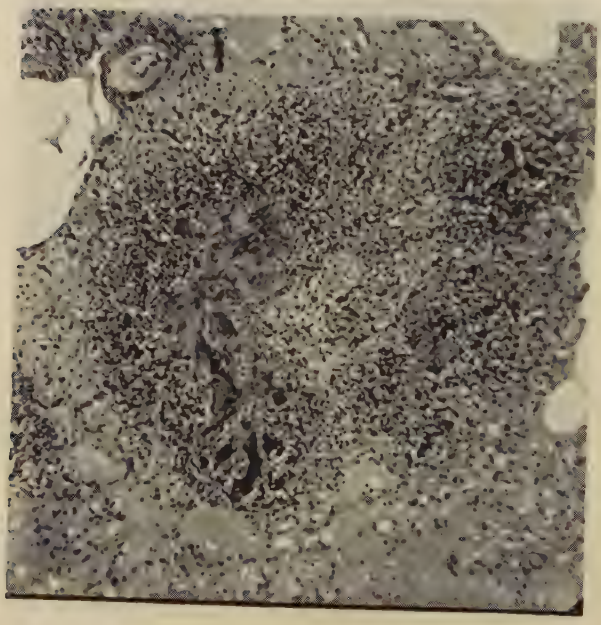

Fig. 5

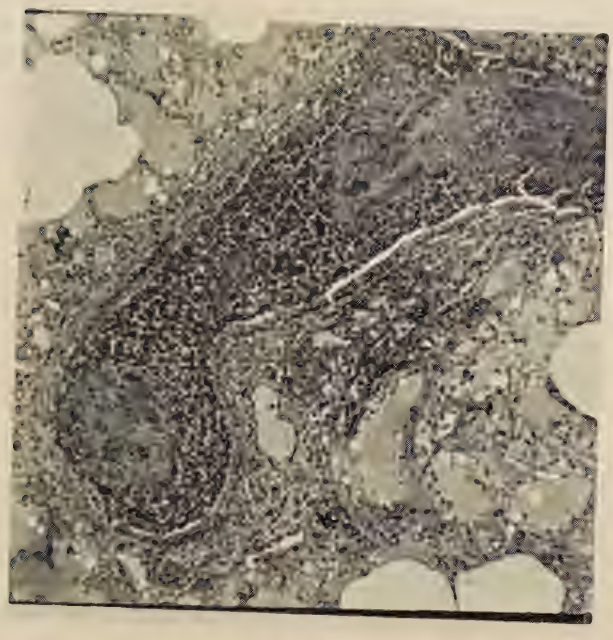

Fig. 6

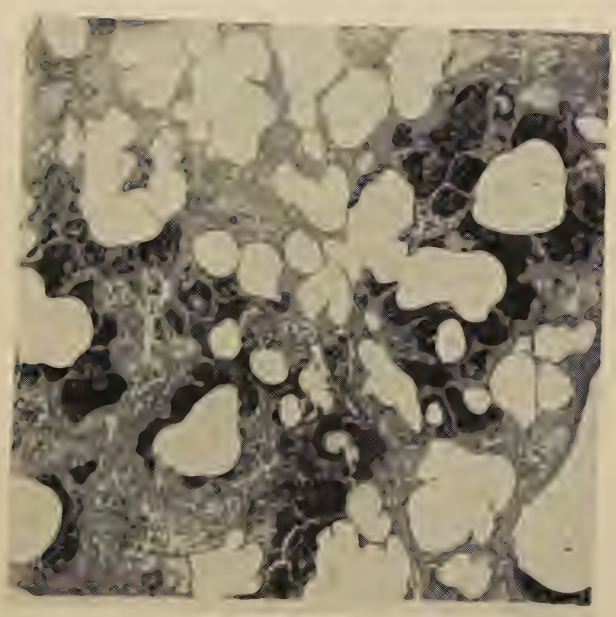

Fig. 7

STUDIES IN ANIMAL TRYPANOSOMIASES.

IV.—The Effect of *Trypanosoma congolense* and *Trypanosoma brucei* on some Inorganic Blood Constituents.

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Trypanosoma congolense and *T. brucei* infections of cattle and sheep cause a loss of body tissues and an increased rate of excretion of body bases, chlorides and phosphates (French, 1937). Continuing the previous biochemical studies on these diseases it was decided to determine what effect the increased excretion of anions and cations had on the level of these constituents in the bloods of the infected animals. The same animals were used in this work as were used in the earlier studies and reference to these (French and Hornby, 1934, and 1935*a*, *b*) should be made for the details of the animals and the various infections. This paper summarises the changes in the blood sodium, potassium, calcium, chlorine and inorganic phosphorus caused by these two species of trypanosomes when cattle, sheep and donkeys were the experimental animals.

What little previous work has been done has dealt chiefly with the effects of the various elements on trypanosomes *in vitro*, or with their influence on the course of the disease. Thus Simons (1918) by using physiologically balanced fluids found that the calcium ion acts beneficially on the vitality of trypanosomes *in vitro*. Kligler and Geiger (1928) showed that, in *T. evansi* infections of rats, the survival time after infection was reduced by the removal of any important cation from the diet. Henderson *et alia* (1931), working with Nigerian cattle in which geophagia had developed as a result of trypanosome infections, recorded that both calcium and sodium supplements had to be fed to stop or prevent the pica They attributed these beneficial effects to the calcium and sodium ions neutralising the acidosis of the disease. Le Roux (1933) also found that the feeding of bone meal was necessary if geophagia was to be prevented in Rhodesian cattle infected with *T. vivax* and *T. congolense*. The only reference I can find dealing with the blood contents of these elements during trypanosomiasis is that of Linton (1930) who recorded no significant variation in the blood chlorine level during *T. equiperdum* infections of rats. On the other hand, Sicé (1930) found a lowering of the chlorine content of cerebro-spinal fluid in nervous cases of human trypanosomiasis.

In the above experiments the effect of the various elements on the course of the disease was determined by restoring the element to a ration deficient in this constituent. The effect of adding salt and lime to rations above that of bare maintenance was studied by French and Hornby (1934) and (1935*a*, *b*) who found that they had no effect on the course of the infection. It was then pointed out (French, 1937)

that, since the cations were required for neutralising the acidosis of the infection, once a sufficiency of minerals for this purpose was provided any subsequent additions could not influence the course of an infection, and that in spite of the disease causing an increased rate of elimination of body bases, chlorides, and phosphates, positive balances could still be obtained if sufficient of these constituents were provided in the diet. It has also been shown (French, 1938) that there is no constant alteration in the total protein content of the plasma, and since the body attempts always to maintain a fixed quantity of base in the body fluids it would seem unlikely that the blood cations would vary appreciably during infection. Unlike the cations the anions in circulation can vary widely in their proportional amounts provided their total quantity remains constant, and it is therefore impossible to predict what changes should take place in the chlorine and phosphorus contents of the blood during infection.

Experimental.—Blood was drawn for these analyses from the jugular vein and immediately a given quantity was measured into 10 per cent. trichloracetic acid. The filtrate was used for the determination of the inorganic constituents. Calcium was determined by Clarke and Collip's (1925) modification of the Kramer and Tisdall (1921) method. Sodium was estimated by Louw's method (1933) and potassium by that of Malan and Van der Linden (1931), whilst chlorine was determined on whole blood directly by the method of Davies (1932). The inorganic phosphorus content was found by the procedure of Fiske and Subbarrow (1925).

Inorganic Phosphorus.—There appears to be no previous reference to the effect of trypanosomiasis on the inorganic blood phosphorus level. In the *T. congolense* infections of cattle and donkeys and the *T. brucei* infections of sheep no change took place in the inorganic phosphorus level with the approach of crisis or death, but in the cases of the two sheep fatally infected with *T. congolense* this fraction decreased steadily. Whether this fall is due to the individual idiosyncrasies of these animals or is characteristic of this disease in sheep has not been ascertained. The constancy of this fraction in the other experimental cases suggests that it is due to causes other than trypanosomiasis.

Calcium.—The changes in the blood calcium content are difficult to interpret. No alteration of the calcium level occurred in the sheep infected with *T. brucei* and which died after an acute infection. Similarly no change occurred in the mild cases of *T. congolense* in Oxen 3046 and 2225 nor in the acute and fatal infections of Oxen 3730, 3622 and 2855. On the other hand a slight lowering is to be noted near crisis in the infections of Oxen 3143, 2785, 2386 and 2864 and in the fatal infections of Sheep M and W.

It is interesting to recall here that a hypocalcæmia has been reported in several acute febrile infectious diseases, and that Mukherjee (1923) found a lowering of the blood calcium in kala-azar (an infection

of man by a related genus of the trypanosomidiae). Large variations are known to occur in work dealing with the blood calcium and these idiosyncrasies are accepted and expected. The variations in the present series are not very striking and no greater than met with in other work on this element. The state and distribution of the blood calcium has not yet been elucidated but it appears to be related to the protein and phosphorus contents. Blood calcium will therefore tend to vary with the proportions of these but the final level will be determined by the extent to which it is being absorbed from the food, mobilised from the skeletal reserves, and excreted from the body.

Potassium.—In every case of infection a fall took place in the blood potassium content, whilst in all controls a smaller fall occurred. In those animals which recovered the blood potassium increased after crisis to a level of approximately that in the corresponding control ox.

In the ox and sheep the red blood corpuscles contain about three times as much potassium as does the plasma (Lintzel, 1931). It follows therefore that any change in the relative proportions of plasma and cells will be reflected in the potassium content of whole blood. If the values given by Lintzel are used, it is possible to calculate what change would be expected from the known anaemic conditions of the animals used in this study. When this is done it is seen that the calculated values decrease in the same way as do the observed values. The figures are not presented for all animals, but in Table VIII are given some of the figures for Ox 3143 which recovered from an acute infection of *T. congolense* and for Ox 3730 which died, as well as the figures for Sheep Umoyo and Tumbo which, though fed differently, died after an acute infection of *T. brucei*.

In Table VIII it is seen that the calculated potassium values are higher whilst the calculated sodium and chlorine values are lower than the experimentally determined values. This difference is probably due largely to the fact that the cell volume determinations were not absolute and that the packed cells still contained plasma.

It appears that the observed fall in the blood potassium content is directly related to the degree of anaemia, and no marked alteration in plasma or cell contents can have taken place.

Sodium and Chlorine.—An increase was observed in both the sodium and chlorine contents of the blood of infected animals, whilst a smaller increase occurred in the controls. A decrease followed crisis paralleling the recovery from the anaemic conditions. As with potassium, Table VIII indicates that the observed increase in these elements is due to the increased plasma content of the anaemic blood resulting from trypanosome infections. Although the observed and calculated values show the same type of change they do not run strictly parallel, but this is largely because absolute values for plasma volumes were not obtained. From these figures it appears that no significant lowering of the blood chloride content occurred near death or crisis due to the acidosis of the infection.

Summary.

1. The inorganic phosphorus content of the blood was unchanged by trypanosome infections in cattle, sheep and donkeys.

2. The blood calcium was unaltered in several cases of trypanosome infections, but showed a tendency to decrease in other cases. Interpretation of the calcium changes is therefore difficult.

3. The potassium content of whole blood fell during trypanosomiasis, the fall being attributable to the anaemic conditions of the blood reducing the proportion of blood cells to blood plasma.

4. The sodium content of whole blood increased during infections, the increase being caused by the increased proportions of plasma in the anaemic blood of the infected animal.

5. Blood chlorine increased during infections up to death or crisis, this increase also being caused by the anaemic conditions of the blood.

It is with great pleasure that I acknowledge the help and interest shown in this work by the Director of Veterinary Services (Mr. H. E. Hornby), and also for permission to publish this paper.

Table I.

Changes induced in some Inorganic Constituents of Blood by *T. congolense* in an Ox on a Maintenance Hay Ration.
(Expressed as mgm./100 ml.)

Animal.	*Weeks after Inoculation.*	*Red Cells (millions).*	*Sodium.*	*Potassium.*	*Chlorine.*	*Calcium.*	*Inorganic Phosphorus.*
T.C. Infected Ox 3143. (Crisis in 19th week).	0	10·0	266·69	34·85	286·86	9·60	6·03
	4	6·1	268·75	29·38	301·39	9·19	5·00
	8	4·2	294·12	26·23	310·58	10·27	5·44
	13	3·6	326·56	24·71	317·57	8·63	5·03
	19	3·6	322·83	24·89	317·02	9·88	4·81
	20	3·6	324·84	24·05	327·09	9·80	5·92
	25	5·2	315·87	26·21	303·71	9·33	5·18
	31	6·4	309·51	27·53	297·12	9·36	4·63
	35	6·8	310·77	28·69	298·74	9·54	5·41
	40	6·9	313·81	28·68	298·68	9·77	5·36
	44	6·4	310·67	31·00	299·32	8·67	5·71
Control Ox 2134	0	9·5	266·69	34·40	286·34	10·03	6·07
	4	8·6	269·69	31·68	284·75	10·01	6·55
	8	8·8	286·67	32·16	290·00	10·82	6·44
	13	7·9	296·68	30·11	298·20	9·45	5·13
	19	7·1	288·44	28·64	296·84	9·98	6·13
	25	7·5	286·11	27·75	302·11	9·98	5·51
	31	8·1	286·86	31·00	280·11	9·79	5·67
	35	8·3	284·18	32·10	290·04	9·87	5·33
	40	7·1	288·33	29·63	286·36	10·03	5·61
	44	7·3	288·44	31·30	285·48	9·99	5·84

Note.—From 20th week the ration was improved by the addition of 1·45 kg. cotton seed daily.

TABLE II.

CHANGES INDUCED IN SOME INORGANIC CONSTITUENTS OF BLOOD BY *T. congolense* IN AN OX ON A MAINTENANCE HAY RATION.
(EXPRESSED AS MGM./100 ML.)

Animal.	*Weeks after Inoculation.*	*Red Cells (millions).*	*Sodium.*	*Potassium.*	*Chlorine.*	*Calcium.*	*Inorganic Phosphorus.*
Infected Ox 2785. (Crisis in 27th week).	0	9·1	271·89	36·20	292·76	10·02	5·98
	4	7·2	285·00	29·45	294·67	9·86	5·55
	8	5·8	304·36	28·54	304·15	10·07	5·63
	13	5·3	318·46	26·11	308·49	9·87	5·06
	19	5·0	319·53	25·86	312·81	9·26	5·71
	25	5·4	315·41	26·11	307·66	10·02	5·33
	31	5·2	322·43	28·63	300·33	10·01	4·99
	35	5·8	314·68	27·16	308·38	9·87	5·12
	40	5·8	320·41	26·68	303·66	9·97	5·35
	44	6·1	308·95	29·88	295·12	10·14	5·01
Control Ox 2863.	0	9·6	273·53	34·00	290·31	9·39	6·00
	4	8·2	282·16	35·01	296·70	9·78	6·40
	8	9·3	280·52	34·12	283·59	9·91	6·10
	13	7·7	286·81	32·33	294·06	9·18	5·40
	19	7·9	294·55	32·51	292·51	9·57	5·51
	25	8·0	298·93	30·87	297·94	9·84	6·15
	31	7·0	304·18	29·35	302·51	9·76	5·76
	35	—	—	—	—	9·84	5·63
	40	6·9	299·33	31·22	305·33	9·87	5·41
	44	7·2	291·58	32·54	298·45	9·57	6·13
T.C. Infected Ox 2855. (Treated at crisis in 12th week but died in 16th week.)	0	9·0	273·18	35·00	285·13	9·39	5·01
	5	6·8	314·23	30·09	300·33	9·12	4·88
	9	4·1	324·31	27·68	316·86	8·79	4·65
	11	2·8	325·52	25·31	323·52	8·86	4·74
	12	2·1	330·19	24·89	336·64	9·00	4·79

TABLE III.

CHANGES INDUCED IN SOME INORGANIC BLOOD CONSTITUENTS BY *T. congolense* IN OXEN ON AN *ad lib.* HAY RATION.
(EXPRESSED AS MGM./100 ML.)

Animal.	*Weeks after Inoculation.*	*Red Cells (millions).*	*Sodium.*	*Potassium.*	*Chlorine.*	*Calcium.*	*Inorganic Phosphorus.*
Infected Ox 2386. (Crisis in 20th week.)	0	8·9	269·63	31·68	280·88	9·81	4·51
	6	4·9	288·68	31·87	314·71	9·85	4·63
	10	4·0	314·42	28·09	321·63	9·12	4·45
	14	3·6	318·42	24·82	326·96	8·94	4·13
	19	3·4	330·66	20·59	333·34	8·96	4·51
	20	3·6	330·84	22·68	327·66	8·59	4·59
	25	4·5	321·00	28·33	329·08	8·88	4·19
	31	4·9	311·88	30·99	310·98	9·41	4·95
	34	5·4	301·41	29·68	301·69	9·33	5·18
	39	5·1	305·12	30·72	304·59	9·33	5·13

TABLE III—*continued.*

Animal.	*Weeks after Inoculation.*	*Red Cells (millions).*	*Sodium.*	*Potassium.*	*Chlorine.*	*Calcium.*	*Inorganic Phosphorus.*
Infected Ox 3730. (Died in 9th week.)	0	8·8	278·41	34·16	274·77	9·22	4·81
	3	7·3	289·36	30·89	296·58	9·36	4·76
	6	4·5	308·64	29·84	311·16	8·90	4·89
	9	3·2	328·50	27·79	324·88	9·45	4·68
Infected Ox 3622. (Died in 12th week.)	0	8·7	274·29	34·90	291·16	10·07	5·16
	3	6·2	296·21	31·79	296·56	10·21	5·05
	5	4·4	303·86	32·13	309·97	10·30	4·96
	7	3·5	312·18	28·64	317·18	10·22	4·91
	9	2·8	322·56	29·32	323·34	10·04	4·85
	11	2·2	322·63	25·45	324·69	10·16	4·97
	12	1·9	330·00	19·99	328·95	10·00	4·92

N.B.—Oxen 2386 and 3730 were fed hay *ad lib.* until crisis and from then Ox 2386 received 1·45 kg. cotton seed daily in addition. Ox 3622 received a good mixed diet.

TABLE IV.

CHANGES INDUCED IN SOME INORGANIC BLOOD CONSTITUENTS BY *T. congolense* IN OXEN ON A GOOD DIET OF HAY AND COTTON SEED. (EXPRESSED AS MGM./100 ML.)

Animal.	*Weeks after Inoculation.*	*Red Cells (millions).*	*Sodium.*	*Potassium.*	*Chlorine.*	*Calcium.*	*Inorganic Phosphorus.*
Infected Ox 3046. (Crisis in 15th week.)	0	10·3	267·77	34·02	282·96	9·60	4·33
	4	6·4	289·63	26·13	296·07	9·63	4·76
	10	5·7	294·68	23·14	304·95	9·67	4·75
	15	5·6	306·88	24·92	308·54	9·34	4·93
	18	7·3	300·22	31·01	297·67	9·80	5·11
	24	8·3	283·11	32·51	289·63	8·68	4·81
	30	9·6	275·31	33·79	277·91	9·18	5·11
	33	10·2	267·77	38·76	266·67	9·76	5·33
Infected Ox 2225. (Crisis in 18th week.)	0	9·6	275·31	31·53	284·33	10·20	5·16
	4	6·4	295·41	26·83	302·16	10·40	5·67
	10	5·3	298·61	25·21	304·65	9·98	5·70
	15	4·3	305·88	26·21	316·07	10·31	5·13
	18	4·8	309·55	24·26	305·71	10·04	5·75
	21	5·7	296·88	26·54	308·45	10·06	5·53
	27	5·7	298·63	27·96	296·47	9·87	4·87
	33	7·4	289·43	28·76	288·38	10·10	5·29
Infected Ox 2864. (Crisis in 9th week.)	0	9·2	277·61	37·90	283·29	9·97	4·80
	3	5·6	290·56	29·59	290·62	9·91	4·96
	5	4·4	305·00	29·17	313·34	9·86	4·88
	9	2·4	331·11	25·84	324·82	9·43	4·65
	9½	2·3	320·54	24·62	320·84	9·38	4·88
	12	4·3	301·76	29·32	312·18	9·49	4·92
	18	6·7	288·95	34·53	292·52	9·54	5·00

N.B.—These oxen were fed 1 kg. cotton seed daily with hay *ad lib.*

TABLE V.

CHANGES INDUCED IN SOME INORGANIC BLOOD CONSTITUENTS BY *T. congolense* IN SHEEP.
(EXPRESSED AS MGM./100 ML.)

Animal.	*Weeks after Inoculation.*	*Red Cells (millions).*	*Sodium.*	*Potassium.*	*Chlorine.*	*Calcium.*	*Inorganic Phosphorus.*
Infected Sheep M. (Died in 18th week.)	0	15·1	271·23	32·00	271·41	9·80	4·72
	2	11·2	291·63	29·12	276·55	9·53	5·01
	4	8·8	287·69	26·31	279·99	9·58	5·00
	6	8·3	290·70	26·06	288·63	8·96	4·53
	8	6·9	308·61	26·06	284·71	9·11	4·15
	11	6·2	312·63	24·00	308·50	8·96	4·50
	14	6·2	312·49	20·66	320·86	9·12	4·21
	17	5·6	318·41	19·87	318·91	8·96	4·22
Infected Sheep W. (Died in 12th week.)	0	14·8	270·41	31·54	268·74	9·47	5·20
	2	11·2	284·32	28·29	274·44	9·18	4·94
	4	9·8	294·88	26·82	305·66	8·90	4·62
	6	6·2	311·64	25·78	297·58	8·91	4·30
	8	6·4	312·61	23·19	286·48	8·78	4·15
	11	5·3	320·41	18·51	308·50	8·93	4·00

N.B.—Sheep W received a sub-maintenance hay ration; Sheep M received a maintenance ration of hay and cake.

TABLE VI.

CHANGES INDUCED IN SOME INORGANIC BLOOD CONSTITUENTS BY *T. brucei* IN SHEEP.
(EXPRESSED AS MGM./100 ML.)

Animal.	*Weeks after Inoculation.*	*Red Cells (millions).*	*Sodium.*	*Potassium.*	*Chlorine.*	*Calcium.*	*Inorganic Phosphorus.*
T.b. Infected Sheep Tumbo. (Died in 12th week.)	0	12·3	285·81	33·72	307·79	9·56	5·07
	3	8·8	303·92	28·75	316·75	8·82	5·33
	6	8·8	316·84	26·66	323·92	10·13	5·00
	9	5·0	324·63	24·77	330·60	9·31	4·73
Control Sheep Majani.	0	12·5	296·78	30·79	303·88	10·31	4·30
	3	12·2	288·32	28·05	307·79	10·47	4·71
	6	12·6	298·71	34·02	305·18	10·79	4·10
	9	—	296·43	33·31	310·27	10·72	4·33
T.b. Infected Sheep Umoyo.	0	15·2	289·62	32·79	279·97	10·65	4·72
	3	10·8	296·43	27·44	299·97	10·15	4·31
	6 (Died)	9·5	313·41	25·00	325·26	10·21	4·91
Control Sheep Dunia.	0	12·8	296·43	32·28	311·69	9·71	4·91
	3	15·3	288·71	36·41	295·71	9·75	4·75
	6	14·2	295·88	32·18	299·18	10·21	4·85
T.b. Infected Sheep Imani.	0	10·0	301·11	35·15	302·22	9·31	4·83
	2	7·8	319·84	27·13	330·47	9·23	4·96
	5 (Died)	6·5	333·73	26·31	336·72	9·81	4·78

N.B.—Sheep Tumbo and Majani received a good ration of hay and concentrates. Sheep Umoyo, Dunia and Imani received a sub-maintenance hay ration.

TABLE VII.

CHANGES INDUCED IN SOME INORGANIC CONSTITUENTS OF BLOOD BY *T. congolense* AND *T. brucei* IN DONKEYS ON A MAINTENANCE RATION. (EXPRESSED AS MGM./100 ML.)

Animal.	*Weeks after Inoculation.*	*Red Cells (millions).*	*Chlorine.*	*Inorganic Phosphorus.*
T. brucei.	0	7·9	285·82	4·18
Infected Donkey No. 3.	2	—	298·61	4·09
	4	4·3	304·76	4·00
	6	2·3	318·92	4·31
	6½	2·1	325·84	4·09
Control Donkey No. 22.	0	7·1	275·52	4·44
	2	7·8	281·66	4·68
	4	7·5	278·37	4·98
T. congolense and *T. brucei.*	0	7·3	303·61	4·23
Infected Donkey No. 17.	2	—	301·35	4·34
	4	7·6	301·97	4·05
	6	6·7	308·44	4·18
	9	3·9	329·63	4·59
	10	2·7	333·84	4·21

Note.—These Donkeys were fed hay *ad lib.*

TABLE VIII.

THE VALUES OF BLOOD SODIUM, POTASSIUM, AND CHLORINE FOUND BY CALCULATION AND ANALYSIS. (EXPRESSED AS MGM./100 ML.)

Animal.	*Week.*	*Sodium.*		*Potassium.*		*Chlorine.*	
		Calculated.	*Found.*	*Calculated.*	*Found.*	*Calculated.*	*Found.*
Infected Ox 3143.	0	251·12	266·69	36·99	34·85	281·92	286·86
	10	291·44	303·55	27·63	27·61	317·04	314·16
	20	288·08	324·84	28·41	24·05	323·28	327·09
	40	276·30	310·67	31·14	31·00	310·12	299·32
Infected Ox 3730.	0	249·44	278·41	37·38	34·16	280·04	284·77
	3	267·24	289·36	33·48	30·89	308·74	296·58
	6	279·68	308·64	30·36	29·84	323·88	311·16
	9	334·28	328·50	27·24	27·79	338·96	324·88
Infected Sheep Umoyo.	0	246·55	289·62	38·55	32·79	278·30	289·97
	3	262·65	296·43	34·65	27·44	298·90	299·97
	6	275·53	313·41	31·53	25·00	315·38	325·26
Infected Sheep Tumbo.	0	261·52	285·81	35·04	33·72	296·84	307·79
	3	278·75	303·92	30·75	28·75	319·50	316·75
	5	281·97	316·84	29·97	26·66	323·62	323·92
	9½	288·41	324·63	28·41	24·77	331·86	330·60

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ABSTRACTS.

FOWL LEUKOSIS*

For a number of years this disease has been increasing in the United States. It is accompanied by a complex of symptoms such as varying degrees of blindness, paralysis of limbs and wings.

During recent years much has been done to determine the true nature of these conditions, especially from the standpoint of ætiology and transmission. Investigations were begun at Iowa State College in February, 1930, and are still in progress. In the beginning the authors were primarily concerned with blindness and paralysis, but early in their studies they were impressed by the frequent occurrence of tumours and tumour-like infiltrations in various organs and tissues of the so-called paralysis cases. Certain histological alterations, varying from slight cellular infiltration to hyperplasia and tumours of the same type of cells, led to the conclusion that paralysis is only one symptom associated with this malady. Later, the close association of leukæmia and erythroleukosis-like manifestations became very apparent, thus making a study of these conditions imperative.

The complexity of the disease has led to variable terminology which expresses in most cases a quite adequate nomenclature for single manifestations but inadequate as a name for the disease as a whole. There is usually considerable overlapping in the various expressions of the disease, so that in each case the nomenclature used pertains to the type of cells in predominance. It would appear that all types come from undifferentiated cells that cannot be classified as long as they remain in the undifferentiated stage. These predominating cells in the lesions are termed hæmocytoblasts, and they possess potentialities towards any of the blood cell types.

A particular case in question can be definitely classified into a definite type when only enough of these cells have differentiated sufficiently to determine their developmental tendencies. In the opinion of the authors fowl leucosis is a more apt term and may be used to designate in common all forms of the disease. It is most commonly differentiated into three types—erythroid, myeloid, and lymphoid. The term erythroleukosis is the term used when primitive cells as hæmocytoblasts and all stages of immature erythrocytes are found in predominating numbers in the peripheral blood as well as accumulation of these in the spleen, liver, and bone marrow. Myeloid leukosis is the term used when such immature granulocytic elements as myeloblasts and myelocytes and hæmocyblasts are found predominating in the peripheral blood, and accumulations of these cells are present in the bone marrow, liver, spleen and kidneys. Lymphoid type and hæmocytoblastosis are the terms used to designate all cases with proliferative accumulations of lymphocytes and hæmocytoblasts in the peripheral nerves, central nervous system, iris, liver, and practically all the visceral organs. Hæmocytoblastoma will be used to designate tumours composed of hæmocytoblasts.

Clinical Manifestations.—The clinical symptoms in this disease complex vary according to the location and degree of involvement of the affected tissues or organs. Cases suffering from lymphoid leukosis or

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hæmocytoblastosis of the nerve type may be observed as a unilateral, bilateral, local or general paralysis. Paralysis of one or both legs or paralysis of one or both wings is most commonly observed. These manifestations may be only slight or may be so severe that the fowl is practically helpless. Frequently, when the legs are involved, common characteristics may be observed such as stiffness, lameness, walking in a stilty manner, with a tendency toward extending the affected leg or legs backward, forward or straight from the body, or one leg may be extended forward while the other is extended backward. In more advanced cases the bird may go completely down, dragging itself about assisted by the wings. When the brain is involved, various lethargic symptoms are observed as well as abnormal head movements, such as a waving motion of the head, throwing the head backward or the beak downward, lateral deviations and in general a lack of ability to hold the head in a normal position. Many cases show paralysis, partial or complete, of many of the visceral organs, the severity depending upon the amount or site of involvement of nerves or nerve plexuses supplying the part. In some cases associated with involvement of the vagus nerve, difficult respiration evidenced by breathing through the mouth has been observed. In general, any variation may be observed, depending upon the nerve or nerve plexuses involved. The onset may be gradual or sudden and the course is variable. The disease may be rapidly progressive or may progress slowly. The present authors have never observed a complete recovery of affected cases, although temporary respites sometimes occur. Emaciated conditions are frequently observed, especially in chronic cases. This emaciation seems to be due largely to difficulty in securing food or difficulty in utilising it. Many cases retain their appetite throughout the course of the disease and many birds that are practically helpless will eat and drink as long as they can reach the feed and water containers.

In the eye cases the iris frequently shows in the beginning a depigmented circle around the pupil. This circle is grey at first or may be only a slightly faded condition and may extend to such an extent that the normal bay colour is almost completely replaced. The process may start in any part of the iris as a small depigmented spot or it may start as a generalised depigmented process throughout the iris, characterised by a faded grey appearance. In many cases the depigmentation appears to start in several places in the iris at the same time, giving the appearance of areas of a normal bay colour interspersed with the areas of faded or grey. The depigmentation is quite irregular and has a streaked appearance, mingling the normal bay colour with that of a pathological grey. The colour changes may vary from a faded bay to a dark or light grey, bluish-grey, or slate colour. In some cases a black coloured tissue appears as a multiple tumourous mass. As a rule both eyes are involved, with one appearing to be more severely affected than the other. However, in many cases only one eye is involved. In a high percentage of cases regular or irregular constriction of the pupil occurs, with impaired vision or even blindness. Opacity of the cornea has been observed in a few cases. The eye type as well as other types of fowl leukosis has a very variable course. The process may spread rapidly or very slowly and in many cases it appears as though the disease has become arrested. The birds usually remain in good health unless blindness occurs or unless eye type cases become complicated with other expressions of the disease. Frequently cases have been observed that were uncomplicated eye cases in the begin-

ning but developed some other type after a variable length of time. The authors have also seen several uncomplicated eye cases that lived two years without any great impairment of health.

Many degrees of clinical manifestations are noted in cases of the greatly varying erythroid type. The most characteristic symptom shown is a severe anæmia with a definite icterus of the skin of the face. The tendency in most of these cases is toward emaciation. However, the condition of flesh in many cases may be deceiving, as it is frequently observed that birds appearing to be in excellent flesh are somewhat œdematous and soft. Positive blood findings in the peripheral circulation of numerous hæmocytoblasts and basophil erythroblasts, presumably precursors of erythrocytes, are frequently encountered and a negative blood picture may not at all times mean that the bird is not affected with some expression of the disease. The course of the disease is also very variable, in some cases progressing rapidly and in others slowly. The disease may appear to be arrested but complete recovery has never been noted. The severity of symptoms is no indication of the extent of involvement. The appetite usually remains good except in the very acute, generalised case and in cases of prostrated birds.

The clinical manifestations of myeloid leukosis are very variable. The onset may be gradual or very rapid and general weakness with diarrhoea is quite common. Marked lameness is frequently found in those cases where marked infiltration in various organs is found on autopsy. The lameness may occur in one or in both legs, but in most cases occurs in both. The skin, comb, and wattles may not show any colour changes, while in some cases varying degrees or anæmia as well as a peculiar pallor may be observed. The presence of large numbers of myelocytic elements in the peripheral circulation serves to make a diagnosis. The tendency in these cases is toward a rather rapid emaciation.

Clinical symptoms in cases affected with hæmocytoblastoma and lymphocytoma tumours and tumour-like infiltrations in various organs vary greatly, depending upon the tissues of organs involved. Lameness has been observed in cases where a tumourous mass was found pressing on a corresponding nerve or nerve plexus. Diarrhœa and emaciation are quite common in the more chronic cases. Many cases in good flesh have died suddenly and upon autopsy been found to be suffering from an enormously enlarged infiltrated liver. Rupture of the liver has occurred frequently in such cases. Pendulous abdomen has been observed in a few cases. Respiratory difficulty was observed in a few cases which upon autopsy showed an involvement of the lungs by lymphocytomatosis and hæmocytoblastosis tissue infiltrations. The symptoms in general are more obscure than in the other types of the disease. A negative blood picture does not necessarily mean the bird is not affected.

Nerve Lesions.—In the nerve type the most characteristic lesions are enlargement of nerves or nodular formation in nerves, with hæmocytoblasts and lymphocyte infiltrations. In many cases the nerves appear only slightly swollen and œdematous, while in others the enlargements may be extreme. The white glistening appearance and irregular striations such as are common to normal nerves are usually less distinct in mild cases and completely disappear from those in badly affected cases. The infiltrated nerve is soft, tumour-like, and a dull grey or fatty yellow colour. The process may be confined to only one nerve or may affect a group of nerves or it may be more generalised, affecting many groups of

nerves. The most severe lesions were those observed in the peripheral nerves and the most common sites were the ischiatic, vagus, brachial, lumbosacral, splanchnic and intercostal plexuses and their branches. The nerve roots and ganglion tissue along the spinal column are very often involved. The pathologic manifestations may be slight and consist essentially of an infiltration of small, intermediate, or large mononuclear cells between the nerve fibres, separating them and accompanied by more or less œdema. In many cases the invading cells are loosely distributed in certain areas of the nerve. In more severe manifestations the invading tissue may almost completely replace the nerve tissue of a nerve or plexus. The tendency is toward a diffuse type of infiltration, although great variation may be observed in different areas of the same nerve. Histologic studies usually reveal an infiltration of variable sized, undifferentiated, mononuclear, basic staining, nongranular cells that are considered on the same basis as undifferentiated embryonic blood progenitors (hæmocytoblasts). They are apparently the same type cells as those usually called lymphoid-like, and have the same characteristics and variations as those found in aleukæmic and leukæmic lymphocytoma, large or small round cell sarcoma and lymphosarcoma. Mitotic figures are frequently observed in cells of the nerve lesions, especially in the intermediate type cells.

Degeneration changes in the nerve fibres vary greatly in the different cases and the extent of degeneration present depends on the amount and intensity of the infiltration.

Spinal Cord Lesions. The lesions in the central nervous system are most often found by histologic examination. In a few cases gross lesions of extensive proportion are observed in the brain and spinal cord. Many of the lesions in the spinal cord originate in the peripheral nerves and extend to the spinal cord. This, however, is not always true as many cases are found with lesions in the central nervous system, with apparently no other nerve tissue involved. In the spinal cord, in addition to the infiltrations accompanying the entrance of a nerve root, there are found focal accumulations of cells more often in the white matter but in some instances also in the central grey. Lesions of this type seem to be limited to the vicinity of the smaller blood vessels. The cells composing these accumulations appear to be hæmocytoblasts and lymphocytes. Degeneration changes in the ganglion cells of the cord are observed only when the lymphoid-like infiltration is so massive as to bring about extensive destruction of the cord tissue.

Brain Lesions.—The lesions found in the brain are always focal. They are frequently found in the cerebrum, cerebellum, optic thalamus and pons. With one exception they were always perivascular rings of compact, densely staining, small lymphocytes. In only one case was a diffuse infiltration found, as observed in nerves and the spinal cord, and this case was characterised by a definite, extensive, tumour-like gross lesion.

Eye Lesions.—The lesions found in the iris and pupil are usually less distinct after death than before. This makes it very desirable to examine the eyes before death whenever possible. In general, the gross lesions in the iris and pupil are not greatly different from those already described under clinical symptoms. The histologic studies reveal that the iris is thickened, œdematous, and infiltrated with the same type of blast stage cells as described in nerve lesions. These appear as small and large, basic staining, non-granular, hæmocytoblasts and lymphocytes. The larger

elements frequently appear as compact areas of cells situated towards the anterior surface. In some eye cases eosinophilic cells seem to be the predominant type, while in others there seems to be a mixture of the two types of cells. The cornea and retina seem to have undergone no change and are regarded as normal. The optic nerve is frequently found to be involved with the same type of cells as described in other nerve lesion cases. The conjunctiva and extraocular muscles contain dense nests of lymphoid-like cells in a few cases. The amount of infiltration in the eye cases varies greatly from slight in some cases to massive in others. There seems to be more correlation between the intensity of symptoms and lesions in this type of the disease than in the other types.

Lesions in Other Tissues.—The lesions in some cases are confined to a single organ or tissue while in others the process is more or less generalised. However, leukæmic and aleukæmic lymphocyte-like tumours and tumour-like infiltrations have been found in practically all of the organs and tissues of the body, the principal locations being in the liver, ovary, spleen, and kidneys. They appear in some cases as diffuse infiltrations; in others as distinct tumours. Various degrees of involvement have been observed, from microscopic foci to those of great proportions. Multiple tumours are frequently found in the liver. This tumourous tissue, which is a greyish-pink or white with occasional yellow colouring is very friable and well embedded in the affected organ. These tumours vary greatly in size from miliary foci to rather extensive formations. In many cases the infiltration is diffuse, causing a slightly or enormously enlarged liver, depending upon the extent of infiltration. The gross appearance of the liver is greatly altered, with a grey colour and a roughened, hobnailed surface and rounded borders. In many cases where death occurs very suddenly the abdominal cavity may be filled with blood due to rupture of a very friable liver. The spleen and kidneys are affected much as the liver and in many cases all three organs show changes. The ovary is very frequently the site of infiltrations. It may be enlarged to several times its normal size, and its normal structure may be obliterated by a mass of soft, yellowish-grey, infiltrating, lymphomatous tissue. In one case, with a large tumourous mass in the ovary, there was a direct extension to the kidney. In another case, an extension from an affected ovary directly into the spinal canal was observed, with lesions in the cord identical with those found in the cord of the nerve type. The same type of undifferentiated cells are found in nerve lesions. In the liver the infiltrations vary from mild peri-portal accumulations to massive infiltrations, which so destroy the liver cells, that in many cases it is impossible to demonstrate the normal liver tissue.

In the kidneys the condition is analogous to that in the liver. In extreme cases the infiltration found between the tubules is so great that the parenchymal elements widely separated or actually replaced. In the spleen the infiltration is found in both the pulp and the follicles.

The tumour-like lesions found in various organs are apparently identical in their gross and microscopic appearance to those manifestations commonly described as round-cell sarcoma, lymphosarcoma, leukæmic and aleukæmic lymphocytoma. The tendency in all cases, regardless of the location of the lesion, is for the pathologic cells to invade and replace the involved tissues. In many cases, histologic examination of some sections indicates that the walls of some blood vessels and capillaries are apparently made up of invading cells.

The classifications of erythroleukosis and myeloid leukosis are based upon blood findings. In some cases with definite blood findings or erythroleukosis lesions were observed in the various visceral organs. In some cases the liver appeared swollen, with rounded edges, and the spleen enlarged and more grey in colour than normal. In many cases tumour-like nodules and diffuse infiltrations of these organs occurred. The bone marrow is always involved in erythroleukosis, being swollen, pale grey or deep red, and more solid in consistency. Microscopically an outstanding feature of erythroleukosis is the stasis of hæmocytoblastic cells in the capillaries. The liver shows at times a great accumulation of these cells in the capillaries, usually intravascular but at times with tumour-like proportions. Very frequently perivascular accumulations and infiltrations occur. The spleen shows an accumulation of similar cells in the pulp and follicles. The bone marrow trabeculæ show numerous lymphocytic and myelocytic foci and in more advanced cases the entire marrow constituents appear as a mass of lymphoid-like cells.

Myeloid leukosis is characterised by the presence of myelocytes in large numbers in the peripheral blood. In some cases, diffuse or nodular infiltrations of soft, loosely arranged, grey or white myelocytomatous tissue occur in the liver, spleen, kidneys and bone marrow.

There are also cases of myeloid leukosis, as diagnosed by blood findings, that show a definite generalised myelocytoma with tumourous formation in the liver, spleen and kidneys. Myeloid leukosis apparently begins with a tumour-like proliferation of myeloblastic and myelocytic elements in the bone marrow, thus flooding the blood with large numbers of myeloid forms, the termination being in accumulation of myeloid cells in the liver, spleen and kidneys.

Blood and Bone Marrow. The positive blood expressions seem to be largely dependent upon the involvement of the bone marrow. When the bone marrow is involved, expressions of myelogenous leukæmia or myeloid leukosis and erythroleukosis appear in the peripheral blood. Eye and nerve types when unaccompanied with bone marrow changes do not show any changes in the blood, but when accompanied by lesions of this disease in the bone marrow may show positive blood findings.

This description has not included all the variations and mixed forms encountered in the study. The organs mentioned as sites of lesions are only a few of those that have been observed to be most frequently affected. It is safe to say that practicallly any organ or tissue may show lesions of this disease.

Transmission Experiments.—In conducting transmission experiments with the disease it was considered of primary importance to have definite knowledge of the origin of the birds used for injection. The birds used in the experiments were hatched at the laboratory from the authors' own breeding flocks, kept solely for this purpose. A definite mating and hatching record was obtained for each chick. The chicks from each hen were separated into a series—that is, one chick for each injection, one for contact, and one for a control. If any cases of leukosis developed in the breeding flock during the experiment the chicks from this hen and mating were immediately removed from the experiment. The controls were kept on a separate part of the farm, with a caretaker who had no contact with other chickens. Three breeds of chickens were used, Single Comb White Leghorns, Barred Rocks, and Rhode Island Reds. The Leghorns were designated as susceptible and resistant since it was believed

that there was a distinct difference in the susceptibility and resistance of that flock. The birds were taken directly from the incubator to the brooder houses which were equipped with screen porches, and at no time were birds allowed to be on the ground.

Transmission by Injection and Pen contact.—A saline suspension made from an infiltrated liver and spleen of a hen that showed no eye or nerve lesions was used as inoculum in these two groups. The tissue was yellowish-grey and lymphoid-like. Histologically the cells were variable in size and shape and were in the undifferentiated blast stage. The bone marrow was severely affected and the blood picture as observed over a period of days was that of erythroleukosis. *Group 1* contained 38 susceptible Leghorns which were placed in the house as soon as taken from the incubator. Nineteen of these were inoculated intra-peritoneally with 2 c.c. of the inoculum when they were ten days old. The remaining 19 birds were left uninoculated, purely for pen exposure contact. *Group 2* consisted of 33 resistant Leghorns. In this group 17 birds were inoculated intra-peritoneally with 2 c.c. of the inoculum when one week old and 16 birds were uninoculated for pen exposure contact.

A table here shows that 60 per cent. of the total 38 birds were affected. In the injected group 14 of the 19 birds (70 per cent.) were affected, and in the pen contact exposure group nine of the 19 birds were affected (50 per cent.). The positive cases included in the table include practically all the previously described types, namely, nerve type, five birds; eye type, one bird; erythroleukosis, four birds; myeloid leukosis, three birds; one case eye and nerve type; four cases hæmocytoblastosis, and five cases lymphocytoma.

This experiment indicated that a transmissible agent was present in the infiltrated organs used as inoculum and that erythroleukosis and myeloid leukosis, hæmocytoblastosis, and lymphocytic infiltrations and tumours may be produced by a common ætiological agent. The disease was transmitted by intraperitoneal injection of affected tissue suspension into young chicks and to a less extent by pen contact exposure. In general there was a difference in time limits between the injected and contact groups, the latter taking a longer time. The earliest case to become affected was in the case of a bird which developed erythroleukosis in 21 days.

Fifteen birds of the same pedigree and age were used as controls, one of these developed myeloid leukosis when one year old and one developed erythroleukosis and eye type at 13 months of age.

Group 2.—This was of a resistant strain of White Leghorns which were inoculated intra-peritoneally with 2 c.c. of the same inoculum used in Group 1. All the birds in this group were hatched April 20th, 1934, and transmission was with unfiltered tissue suspensions.

A table here shows that 24 per cent. of a total of the 33 birds were affected: in the injected group, six of the 17 birds, or 30 per cent. were affected, and of the contacts two of the 16 birds or 12·5 per cent. The positive cases included erythroleukosis, three birds; nerve type, one bird; eye type, one bird; lymphomatosis, one bird; and hæmocytoblastosis, two birds.

The birds in this experiment were inoculated with the same inoculum as Group 1 and were of similar pedigree from the authors' breeding flock, and were cared for in the same manner. The controls of this group, consisting of 15 birds showed no evidence of the disease.

Experiment 2 (*Transmission by Injection and Pen Contact*). — A saline suspension made from two markedly enlarged and infiltrated ischiatic nerves was used as injection material for Groups 3 and 4.

The cockerel from which this tissue was obtained showed a complete paralysis of both legs which had existed for a considerable time. He was extremely emaciated and weak. The blood smears over a period of two weeks had been normal and upon autopsy no lesions were noted except in the ischiatic nerves, which were enlarged, œdematous, soft, yellow-grey in appearance and had no visible striations.

Group 3 was composed of 30 Barred Rocks from the authors' own breeding flock of which 15, when one week old, were inoculated intraperitoneally with 2 c.c. of a nerve suspension; the remaining 15 were left uninoculated purely for pen exposure.

Group 4 consisted of 30 resistant Leghorns, of which 15 were inoculated and 15 were left uninoculated for pen exposure contact. The inoculations were made with the same material as was used in Group 3 and the birds were inoculated in the same manner when one week old.

Group 3.—A table shows that there were 18 positive cases among the 30 birds, or 60 per cent. In the injected group 11 of the 15 injected were positive or 75 per cent.; in the contact group 7 were positive out of 15, or 47 per cent.

The positive cases included practically all the previously mentioned manifestations of the disease; erythroleukosis, three birds; myeloid leukosis, one bird; hæmocytoblastosis, three birds; nerve type, five birds; eye type, three birds; lymphocytoma, two birds; one combination of eye lymphocytoma and nerve type.

This experiment indicated, like experiment 1, that the transmitting agent was present in the infiltrated nerve used for injection and that hæmocytoblastosis, lymphomatosis, nerve type, eye type, erythroleukosis and myeloid leukosis may be produced by a common ætiological agent.

The controls of this experiment developed two cases out of 15 birds —one myeloid leukosis and one eye type. These two cases, however, did not develop until about 14 months of age.

Group 4.—Group 4 consisted of 15 resistant Leghorns of which 15 were injected intraperitoneally with 2 c.c. of the same inoculum as used in Group 3. Table 4 gives the data on this group. These show that ten birds out of the group of 30 (33⅓ per cent.) became affected. In the injected group seven or 47½ per cent. were affected and in the contact group three, or 30 per cent., were affected. The various manifestations noted were erythroleukosis, one bird; myeloid leukosis, one bird; hæmocytoblastosis, one bird; lymphocytoma, two birds; nerve type, five birds.

These birds were inoculated with th same inoculum as Group 3 and, it is to be noted that 50 per cent. of Group 3 became infected, whereas only 33⅓ per cent. of Group 4 were affected. These indications seem to express a variance in resistance by various strains of birds.

The control group consisting of 15 birds remained healthy throughout the experiment.

Experiment 3 (*Transmission by Injection and Pen Contact.* — A tissue suspension in saline solution was made from typical ovarian tumour. This pathological tissue was yellow-grey, soft and lymphoid-like in character. Histologically the cells composing the tumourous mass were hæmocytoblasts and lymphocytes. There were no other lesions in the bird and blood smears were normal.

This group consisted of 30 susceptible Leghorns, of which 15 were inoculated intraperitoneally with 2 c.c. of the above inoculum when ten days old. The remaining 15 birds were left uninoculated, purely for pen exposure contact. Table 5 gives the data on this group.

Group 5.—In this group 17, or 61 per cent. of the entire 30 were affected, of which 11, or 78 per cent., of the 15 inoculated were positive and six or about 40 per cent. of the birds for pen exposure contact were affected.

The various manifestations recorded were: seven lymphocytoma; two haemocytoblastosis; one myeloid leukosis; three erythroleukosis; two nerve type; one eye type and two were mixed types.

The control group of 15 birds remained comparatively free; only two eye type cases were observed during a period of 18 months.

Group 6.—This consisted of 30 Rhode Island Reds and were inoculated with same material as used in group 5. This group was used primarily as a comparison of susceptibility or resistance between a heavy and a light breed. In this group 16 birds were inoculated intraperitoneally with 2 c.c. of the same inoculum as used in Group 5. The remaining 14 birds were used for pen exposure contact.

Transmission with Unfiltered Tissue Suspension.—A table shows that 16 birds of the 30 became affected. In the injected Group 2, of the 16 birds injected or 70 per cent. became affected while in the pen exposure contact group only five out of 14 or 40 per cent. were affected. It is to be noted that of the number of affected birds, one had erythroleukosis, two myeloid leukosis; two eye type; two nerve type; seven lymphocytomatosis; and 2 hæmocytoblastosis. There appears to be no appreciable difference in the susceptibility or resistance of the two breeds used in Groups 5 and 6. Of the controls of the group, 15 in number, no cases developed over a period of 18 months.

Experiment 4 (*Transmission by Injection of Filtrates and Pen Contact*).—A saline suspension of a large tumourous mass of the kidney of bird S was passed through a Berkefelt 3N filter. The tissue was histologically that of a hæmocytoblastoma and the birds showed no other lesions.

Two c.c. of the filtrate were injected intraperitoneally into 21 susceptible Leghorn chicks when ten days old. Eight birds in this group were left uninoculated for pen exposure contact. Table 7 gives the data on this experiment.

Group 7.—It is to be noted from Table 7, seven birds of the 29 became infected or slightly more than 20 per cent. Of this number, six were of the injected group and only one of the contact group. In the affected group, two were affected with erythroleukosis; two with lymphomatous tumours or tumourous infiltrations; one nerve type; one hæmocytoblastosis; and one with the eye type. The percentage of positive birds was much less with filtrates than with tissue suspensions. It appears, however, from the number affected, that the disease is transmitted by filtrates of infected tissues. In this experiment as with tissue suspensions, many manifestations of the disease occurred.

Fifteen controls used in this experiment failed to show any indication of disease during an 18 months' period which would tend to further strengthen the possibility of transmission by filtrates and lessen the possibility of spontaneous infections.

Group 8 (*Seitz filtrates*).—This group was composed of 27 susceptible Leghorn chicks. In this group 21 chicks were inoculated with 2 c.c. of

the same tumourous material as used in group 6, except this material had been passed through Seitz filters instead of a 3N Berkefeld. The injections were made intraperitoneally when the chicks were ten days old, six chicks being uninoculated for pen exposure contact. Table 8 gives data on this experiment.

In Group 8, seven of the 27 birds, or 25 per cent., became affected. It is to be noted that all the affected birds were from the injected group and that the following manifestations were present— erythroleukosis, one bird; myeloid leukosis, two birds; nerve type, one bird; lymphocytoma, three birds. There seems to be very little difference in use of Seitz filters or a Berkfeld 3N filter as far as percentage of transmissible cases were concerned.

The controls of this group, ten in number remained healthy with the exception of two eye type cases which developed at about eleven months of age.

Experiment 5 (*Transmission by Litter Exposure Contact*).—The house used for this group had been previously used for transmission groups and had housed numerous birds showing the various manifestations of fowl leukosis. The litter which was ground cobs and the droppings was not removed from this house during the previous experiments and was allowed to accumulate for two years. The litter was about six inches deep in the house and the only preparation of this house was to rake the litter and dry it.

Thirty chicks of a susceptible strain were placed in this house when taken from the incubator on April 20th, 1934. Table 9 gives data on this group.

Group 9.—The tabulations were made over a period of 19 months and birds that were not autopsied appeared in perfect health. Differential smears as well as blood counts were performed on all birds classified as negative before they were allowed to be put in this classification. It is to be noted that 15 birds or 50 per cent. of the entire group became affected during the 19th months period. It is also interesting to note that as in the other experiments practically all the various forms or manifestations occurred. The following different expressions accurred—lymphomatous tumours and infiltrations, three birds; myeloid leukosis, two birds; erythroleukosis, two birds; nerve type, six birds; eye type, one bird and one bird with a combination of eye type and hæmocytoblastosis.

This experiment indicates that the infection may be quite easily transmitted by contact with contaminated litter or surroundings. This is important to know in investigating any valuable control measures. It also indicates infection may remain in houses or litter for at least two years and probably for a much longer time.

The controls of this group were kept for 19 months and of the group of 15 birds, one bird developed a lymphoid-like tumourous mass in the ovary and one a complete paralysis case with infiltrations of the ischiatic nerves.

Experiment 6 (*Transmission through the Egg*).—It seems of prime importance and interest to determine whether chicks hatched from eggs laid by affected birds will inherit the disease. The fact that lesions are sometimes found in the ovary, oviduct and testic, suggests that the disease may be spread to their progeny before the function of the reproductory organs is completely checked.

There are many difficulties in securing eggs from paralysed hens as paralysis causes a cessation of egg laying in most cases. The selection of birds suffering with internal lesions is a matter of guess so far as clinical diagnosis is concerned. In cases where a definite diagnosis can be made from examination of the blood, other difficulties arise as most cases in which the blood is involved fail to reproduce. In cases where one secures a goodly number of eggs it is found that most fertile eggs die during the period of incubation.

In this experiment we were able to secure 18 chicks, of which four developed various manifestations of the disease over a period of 18 months.

This experiment was carried on with such a small group of birds that no definite information was obtained.

Summary

The term fowl leukosis is used to include in common the group of transmissible, leukæmic, aleukæmic and leukæmic-like diseases of fowls. The disease in all its expressions may be transmitted to healthy chicks by injections of tissue suspensions of affected organs or by injection of cell-free filtrates or may be transmitted by direct pen contact or by rearing chicks on contaminated litter.

The injection of a suspension made from one type apparently produces all the various types considered as expressions of this disease. Different suspensions experimentally produced similar results. Neuro-lymphomatosis associated with eye lesions, hæmocytoblastosis, lymphoid, erythroid and myeloid types of leukosis are different expressions of the same disease and are transmitted by a common ætiologic agent.

The clinical course is variable, in some cases comparatively short and in others extending over a period of months. No complete recoveries occur. There seems to be a definite inherent difference of susceptibility and resistance by different birds and birds of different strains. The evidence seems to suggest that the disease may be transmitted through the egg to a limited extent. The most important methods of control are proper sanitation and use of breeding stock from resistant sources.

PSEUDO-TUBERCULOSIS OF RODENTS*

By Paul Boquet

Notwithstanding the researches on this subject which were carried out by Nocard and others last century the disease remained badly defined for a long time. Some authors even considered it a connecting link with *B. tuberculosis* when encountered in the lesions of so-called pseudo-tuberculosis encountered in guinea-pigs and some other animals.

The guinea-pig is the animal most susceptible to the disease, but its occurrence has also been described in the common fowl, turkey, and other birds, and also in the ox, horse, monkey, and even man. Outbreaks of the disease have often been observed in guinea-pigs, most frequently in winter though the disease may occur at any time of the year. One can recognise different types, such as hyper-acute or septicæmic causing death in 48 hours, an acute type taking the form of a progressive cachexia, and a chronic type lasting for some weeks and sometimes terminating in recovery. The septicæmic form is not readily recognised by its symptoms but in the progressive type with pseudo-tuberculous lesions the disease cannot be overlooked. Loss of condition is the principal symptom and post-mortem examination reveals innumerable white round lesions in the liver and spleen which in appearance resemble small tubrcles. Along with these the mesenteric glands may be enlarged, sometimes even to the size of a pigeon's egg, and purulent.

The organism is easily obtained on the common mediums from the fresh lesions. On agar is grows in the form of short bacilli, singly or in chains, and in broth as chains.

The organism tends to be polymorphic and different names have thus arisen, such as *Bacillus pseudo-tuberculosis rodentium (Pfeiffer)*, *Streptobacillus of pseudo-tuberculosis* (Dor), or *Pasteurella pseudo-tuberculosis* (Topley and Wilson).

Considerable variations in the form of the organism occur in different media. The author found that filamentous forms develop most readily on agar to which 3 per cent. of chloride of sodium has been added.

The cocco-bacilli of the disease are stainable by Gram's medium and they form neither flagella nor spores. Isolated colonies on agar are rounded (bombées), slightly milky but translucent. They continue to extend a little, to become confluent, and old cultures have a disagreeable odour. In broth containing 6 per cent. of ox bile the organism grows just as in plain broth.

In gelatine the organism forms a greyish growth along the line of puncture, but without liquefaction. It forms small white colonies on solid serum, which it does not liquefy. It does not coagulate milk or form indol. It has no hæmolytic effect on blood.

The strains which the author employed in from 24 to 48 hours at 37° C. produced acid without the formation of gas in glucose, levulose, maltose, galactose, gycerine and mannite. It had no effect on lactose, saccharose and amidon.

Some of the characters which have just been mentioned belong also to the Plague bacillus. The latter has the same cultural characters in broth, agar, and gelatine. The two organisms are, however, easily distinguished by the fact that the bacillus of pseudo-tuberculosis grows on

*Annales de l'Institut Pasteur, Vol. lix, p. 342, 1937.

potato, which, on the other hand, is not suitable for the culture of the bacillus of Yersin.

The organism of pseudo-tuberculosis presents the following distinguishing features. In the first place it forms flagella in cultures at 18° to 20° C., and at 37° it develops two variants which by their characters recall the "smooth" and "rough" variations described by Arkwright (*Jour. Path. and Bact.,* Vol. xxxiii, 1920, p. 358, and Vol. xxxiv, 1921, p. 36. Also *The Lancet,* Vol. 212, 1027, p. 13).

After the first description by Malassez and Vignal and Pfeiffer's work this organism was considered as non-motile. Klein, however, (1899) described it as having flagella, but Kossel and Overbeck (1902) described it as non-motile in cultures at 37° C., but motile in cultures at 20° C. It was also described at motile by Arkwright (1927) and by Lewinthal (1930).

For the study of this character the present author used twelve strains of the organism. At 18° to 20° C. in Martin's broth in the majority of the strains a uniform turbidity was produced, but the organisms remained immobile in the medium. In the same medium with 10 per cent. of horse serum for an ordinary broth it showed slight motility. In cultures of the same media the organisms were non-motile. The tendency to motility varied with different strains.

Pathogenic Properties.—The rat and the mouse resist infection with pseudo-tuberculosis but the guinea-pig is very susceptible. The inoculation of 1/10th of 1 c.c. of a culture in broth in 24 hours in a guinea-pig weighing 400 grammes causes a local swelling, sometimes as large as an almond. Rapid emaciation follows and death in 10 to 15 days. In a guinea-pig inoculated subcutaneously with a very virulent culture it proved fatal in a dose of 0·00001 c.c. After continued cultivation for a year the virulence diminishes greatly.

The guinea-pig can be easily infected by the injection of a very small amount of virulent strains into the plantar cushion or into the skin. This causes extensive ulceration and enlargement of the regional lymphatic glands. The infection finally extending to the spleen, liver and lungs, and death occurs in one or two weeks. The guinea-pig is equally sensible to intraperitoneal inoculation.

The histology of the lesions is summed up as follows: —

The granulations in the liver and spleen appear as small circumscribed foci surrounded by a zone of reacting histiocytes. At this level the leucocytes, which are principally lymphocytes and plasma cells, are scanty. Sometimes there are very small necrotic foci without any appreciable surrounding inflammatory reaction.

The zone of leucocytic infiltration (polynuclear lymphocytes, plasma cells) and the reaction by histiocytes is specially extensive in the large nodules in the liver. These tend to become surrounded by a fibrous capsule.

In sections of spleen from two guinea-pigs inoculated subcutaneously with 4 c.c. of a feebly pathogenic strain two characteristic giant cells were seen at a distance from the specific lesions of pseudo-tuberculosis in the substance of the spleen, without any inflammatory reaction around.

Toxicity and Reactional Properties.—De Blasi (1908) and Dessy (1925) failed to find in cultures of the organism any substance toxic for the guinea-pig. On the other hand G. Ramon in 1925 pointed out that

horses are very sensitive to intravenous inoculation with culture filtrates.

The present author found that filtrates of culture injected into the peritoneum of guinea-pigs were non-toxic.

Culture products were found to be toxic for animals infected with the disease when inoculated into the peritoneum, death occurring in 18 hours, while control guinea-pigs were not infected. The same toxic products had no effect on tuberculous animals.

Immunity.—In view of the close immunological connection which unites the cocco-bacillus of pseudo-tuberculosis to Yersin's bacillus many experiments in cross vaccination of these organisms have been carried out.

The author's experiments in immunisation were carried out with strains which had been maintained for some years on artificial media and were avirulent. Guinea-pigs vaccinated by intra-peritoneal inoculation with 2 c.c. broth culture of an attenuated strain resisted a subcutaneous or intradermic test made 17 days afterwards with a virulent dose of 0·1 c.c. After 48 hours a thickening of the cellular tissue and a swelling of the neighbouring lymphatic glands developed in the guinea-pigs tested subcutaneously. In some days this was converted into a small abscess which was afterwards gradually absorbed. In guinea-pigs tested by way of the dermis the skin in 24 hours became the site of an œdematous infiltration and acute inflammation; later this focus was the seat of necrotic degeneration. Cicatrisation was complete in 10 to 15 days. The majority of the animals vaccinated remained free from any visceral lesion.

On the other hand in fresh guinea-pigs used as controls, which had been inoculated with the same virulent strain, infection developed rapidly and was attended by the formation of a huge subcutaneous abscess or a cutaneous ulcer complicated by inflammation of the regional lymphatic glands. The animals died after one or two weeks with characteristic lesions of pseudo-tuberculosis in the liver and spleen.

Guinea-pigs infected experimentally with nodular pseudo-tuberculous lesions, and animals vaccinated and tested by a double inoculation of the cocco-bacilli of Malassez and Vignal showed an appreciable immunity against the second anthrax vaccine.

This paraspecific develops parallel to a specific immunity. In vaccinated guinea-pigs it is manifested even in the absence of any macroscopic lesion in guinea-pigs vaccinated against tuberculosis by means of BCG.

Conclusions.

Cultures are non-motile at 37° C. but at 18° to 20° C. show distinctly active movements. This motility is ascribable to flagella and it is more or less marked according to the strain; old strains of cocco-bacilli cultivated for several years as 37° C. on artificial media remain non-motile when cultivated at 18° to 20° C.

Smooth and rough cultures have been isolated but owing to the great instability of the colonies in morphological appearance it was considered better to substitute for the terms rough and *smooth*, *homogeneous* and agglutinable in normal saline, which expresses one of the principal physical characters of disassociated elements.

The biochemical, pathogenic and antigenic properties of the two variants are equally distinct. The homogeneous variant attacks sugars more actively than the agglutinable variant; on the other hand the latter degrades glycerine more rapidly.

The homogeneous variant of a very pathogenic strain, developed at 37° C., was found to be more virulent for the guinea-pig than the corresponding agglutinable variant. However, the processes of attenuation and virulence are independent of the phenomenon disassociation. Culture at 20° C. seems to increase the pathogenic properties of the preceding strain.

Both the homogeneous and the agglutinable strains are motile at 20° C. At 37° on agar media they are non-motile. In broth the homogeneous variant was unstable.

Although they may be non-ciliated at 37° the two variants nevertheless are provided with the flagellar antigen. This antigen is less abundant in cultures of the agglutinable variant than in cultures of the homogeneous variety. Cultures at 20° C. of both variants are richer than cultures at 37°.

The dispersion of cocco-bacilli in the healthy organism and in the immune organism is in all respects comparable to the dispersion of tubercle bacilli in primary infection or superinfection, such as was described by Allen Krause with H. S. Willis, U.S.A., and by A. Boquet in France.

Virulent strains of the bacilli inoculated under the skin of the guinea-pig after a short interval are spread by way of the blood and lymph vessels towards the deeper organs where they determine the usual lesions of pseudo-tuberculosis.

A transient bacillæmia can be detected during the first hours of the infection.

Avirulent cocco-bacilli spread less rapidly and are destroyed in the organs. They confer on the guinea-pig a specific immunity which is manifested in a test of virulence by partial or total blockage of the germs of super-infection at the place where they were inoculated, and by the gradual destruction of the elements which succeed in penetrating into the visceral organs.

The immunity thus conferred by the avirulent organisms is accompanied by a paraspecific immunity in regard to anthrax, similar to that which has been observed in tuberculous animals.

Parallel to the pseudo-tuberculous infection there is developed an allergic condition which can be made manifest by the local and general reactions to sterilised cultures in liquid media. The cultures in broth at 20° or at 37° are nontoxic for the rabbit and guinea-pig, but they are toxic for guinea-pigs infected with pseudo-tuberculosis. Besides they are capable of preparing and dechaining the phenomenon of Sanarelli-Shwartzman in the rabbit.

The Bibliography at the end of the article extends to two complete pages.

EXPERIMENTAL RESEARCHES REGARDING AUJESZKY'S DISEASE OR PSEUDO-RABIES*

THE present article continues the account of the disease which the authors published in the same journal in 1935. In the researches described in it eight strains of the virus were used, *viz.*,

Ox Pasca	Strain I
Sheep Budapest	II
Swine Aujeszky	III
Swine Holland	IV
Mad Itch (Shope)	V
Ox Holland	VI
Cat Budapest	VII
Ox Manninger	VIII

Throughout the present abstract Au. D. is used for Aujeszky's Disease.

Behaviour of the Virus with Regard to Chemicals.—Its resistance and power of diffusion were previously studied by Zwick and Zeller and later by Remlinger and Bailly, and the present authors have therefore carried out only a few experiments bearing on these matters. Whereas Remlinger and Bailly found that the Au. D. virus reacted in the same way as the virus of rabies towards heat, desiccation, ether and glycerine, the present authors found certain differences in the strains which they used. They found that the virus of Au. D. was less resistant towards heat than the rabic virus; heating thick emulsions of the brain of diseased animals to 60° C. killed the virus in about half an hour, and at 70° in ten minutes. Remlinger and Bailly even after drying for 193 days over caustic alkali found Au.D. virus still virulent, whereas the present authors found all the strains examined by them already avirulent at the fourteenth day. The fixed rabies virus of the Institute in Tangiers, according to the authors mentioned, under desiccation lost its virulence in five to six days. The present authors found that their fixed virus under the same procedure was still active. The principal difference, however, between the Au. D. and the rabies virus was found in regard to the effect of glycerine. Whereas their numerous strains of street rabies and fixed virus in a mixture containing glycerine and common salt in equal proportions at 0° C. remained virulent up to ten months and often longer, and at 6°—8° C. over a year (in one case up to two years), at the same temperature in the same solution they were not able to keep the Au. D. virus longer virulent than three months. The strain V (Mad Itch Shope) regularly lost its virulence after three to four weeks, whereas Remlinger and Bailly were able to recognise virulence even after 400 to 500 days.

The present authors were able to confirm the observations of Remlinger and Bailly in regard to the diffusion of the Au. D. virus and also of the rabies virus into surrounding glycerine, and also into a normal brain suspended in glycerine with but not touching an infected brain. The present authors found that the Au. D. virus diffused more rapidly and more frequently than the virus of rabies in glycerine and into the normal brain.

With regard to the behaviour of Au. D. viruses towards chemicals they formed practically the same opinions as Zwick and Zeller. The

*By Franz Gerlach and Fritz Schweinbürg. *Zeits. für Infeck. Krankh. der Haus.* Vol. L, pp. 86-128.

viruses were killed in five minutes in 5 per cent. antiformin, 3 per cent. carbolic acid, and 2 per 1,000 sublimate solution. A 1 per 1,000,000 sublimate solution and a 1 per cent. antiformin solution left the virus alive for more than an hour.

Like Remlinger and Bailly, they found that in moderately thick Au. D. emulsions long continued sharp centrifuging did not succeed in making the top stratum of clear fluid free from virus; in contradiction with this, the same process was always successful with rabies and herpes viruses.

Filtrability of the Au. D. Virus.—The virus of Aujeszky's disease belongs to the group of filtrable viruses. The authors have repeatedly passed the different strains of virus through Chamberland and Berkefeld filters, thick emulsions of the strains having been first passed through cotton-wool and then through filter paper. Cerebral inoculation of rabbits with the filtrates have generally had positive results, but the incubation period was often prolonged to from two to four days, no doubt caused by the arrest of a considerable proportion of the virus in the filter. Like Remlinger and Bailly, the authors did not succeed in passing the virus through Berkefeld V and collodion filters.

This determination of the filtrability was in disagreement with the views of the older writers, especially with experiments of Zwick and Zeller, but in agreement with the experiments of Remlinger, Bailly, Shope and others.

Results of Attempted Transmission of the Virus to Experimental Animals.—The strains differ in respect of incubation period and transmissibility; many of them considerably. In respect of the clinical picture in laboratory animals and serology they do not differ. The testing of the virulence of the strains for rabbits was carried out by different methods of infection, because it was found that the virulence (expressed in the smallest effective (disease-making) dose) in repeated passages in rabbits with the same method of infection was always the same. The incubation period also always remained the same in the course of continued passages of the same strain. It was only in the most extreme but still effective dilutions that a prolongation of the incubation period occurred.

The results were completely different with guinea-pigs, but are not discussed.

For testing the virulence on rabbits, intra-cerebral, subcutaneous, intra-muscular and intra-peritoneal methods were employed. With inoculation of Strain I in dilutions of 1 : 5,000,000 and 1 : 500,000 the original cerebral incubation of three to four days was lengthened to 12 or 13 days. Higher dilutions than 1 : 500,000 did not give regular results, and only in about half of the infected rabbits.

Subcutaneous inoculation with the same virus regularly infects in a dilution of 1 : 100,000 with an incubation period of four days, but in stronger dilutions the result was negative.

Intra-muscular inoculation had the same result as subcutaneous, but in a dilution of 1 : 50,000 there followed only a prolongation of the period of incubation from five to seven days.

The limit of intra-peritoneal inoculation lies at 1 : 100,000 and the incubation period gradually increases to ten days. The degree of the dilution and the method of infection caused no difference in respect of the clinical symptoms.

Under similar conditions for strain II the limit of dilution for subcutaneous infection was 1 : 100,000, for intra-muscular infection 1 : 50,000, and for intra-peritoneal infection 1 : 10,000, and in the latter dilutions there was again a prolongation of the period of incubation by one to four days.

Another strain, No. VI, was still more virulent by cerebral injection, was effective by subcutaneous inoculation of rabbits to 1 : 10,000,000, and intra-muscularly to 1 : 300,000.

In strain VIII also the dilution for cerebral and intra-muscular inoculation could be carried to 1 : 1,000,000.

With the strains used the smallest effective dose for producing the disease by cerebral inoculation varied from 1 : 10,000,000 to 1 : 100,000; by intra-muscular inoculation between 1 : 3,000,000 and 1 : 5,000; subcutaneously between 1 : 3,000,000 and 1: 5,000; but the effective power by the subcutaneous and intra-muscular routes did not always run parallel. Ultimately the power of infection by the intra-peritoneal route was between 1 : 500,000 and 1 : 1,000. The smallest dilution capable of producing the disease by intravenous and intra-cutaneous virulence was about the same as for subcutaneous infection. The differences in virulence between the different strains were thus considerable even in rabbits. Nevertheless no distinction could be made in the clinical picture produced by the different strains; that is in inoculation with all the strains the condition picture was the one previously described. For rabbits also the incubation period with all strains was two to four days by cerebral, and two to six days by intra-muscular or subcutaneous infection.

As Shope, and later Remlinger and Bailly, pointed out, the behaviour of the Au. D. virus in passage in guinea-pigs shows the rabbit is the best experimental animal for the study of Au. D. and for this purpose guinea-pigs are less suitable, but in the works of these authors complete evidence for these opinions was not furnished. Shope assumes that the brain of the rabbit contains about ten times as much of the Au. D. virus as the guinea-pig's brain, that for subcutaneous infection the rabbit is about one hundred times more susceptible than the gunea-pig, but that in guinea-pig passages a diminution in virulence occurs. The present authors in extensive experiments were able to confirm these opinions but they observed some points of special interest.

Other Methods in Infection.—Intravenous infection, using Schweinbürg's method for rabies infection, is in rabbits 100 per cent. effective in all passages. In guinea-pigs quite regularly the disease is produced by this method in the first passage, but in further guinea-pig passages the infectivity rapidly declines, about one passage sooner than by the intra-peritoneal method.

In rabbits corneal infection was apparently without limit 100 per cent. effective. In guinea-pigs it was the same as the subcutaneous method, but lasted in some cases one or two passages longer.

In rabbits and guinea-pigs intra-ocular inoculation had the same effect as the corneal.

Intra-cutaneous inoculation is always infective with the production of a swelling in rabbits. With guinea-pigs the results were the same as by the subcutaneous method and there was only one strain (No. V) in which infection by the intra-cutaneous method failed.

Plantar experiments (as with herpes virus) had in rabbits and guinea-pigs the same results as intra-cutaneous infection. With all strains friction applied to the intact skin by means of tampons of wadding saturated with thick virus emulsion remained without effect on rabbits and guinea-pigs.

Rubbing into the carefully shaved skin without causing bleeding or any apparent loss of substance also had negative results with all the strains. Rubbing into the roughly shaved skin, with resulting bleeding and small loss of substance caused infection in about 60 per cent. of the experiments. When an interval of 24 hours was allowed between the shaving and the application of the virus the result was negative.

Several drops of a concentrated suspension of the virus were dropped into the intact eye and the eye-lids were then closed for several minutes, but this failed to produce the disease.

Rats and mice reacted in the same way as guinea-pigs. The period of incubation and the smallest effective dose are the same for these three species. The decline in the strength of the virus in continued passages in rats and mice is the same as in guinea-pigs but in rats some strains by all methods of infection require one or two passages more before the virulence disappears.

Summary.—In the authors' experiments on experimental infection in small laboratory animals the virus of Aujeszky's disease showed the following properties. In rabbits it may be transmitted for an unlimited number of passages without change of virulence or period of incubation. The smallest pathogenic dose in the separate strains was between 1 : 10,000,000 and 1 : 100,000 by cerebral inoculation, and between 1 : 3,000,000 and 1 : 5,000 by intra-muscular and subcutaneous inoculation. The period of incubation with the different strains after cerebral inoculation with thick emulsions lay between two and four days, and after inoculation with the most dilute dose the period of incubation was from 30 to 40 days.

Rabbits infected by cerebral inoculation with concentrated emulsion always died; the greater the dilution of the virus the longer the animal survived. Rabbits infected by other methods of infection more frequently survived than those infected cerebrally; especially after intra-peritoneal, intra-cutaneous and intravenous infection 50 per cent. of the animals may survive. Here also the number of surviving rabbits increased with the degree to which the infecting virus was diluted.

In guinea-pigs, rats, and mice the virus with continued passages behaves differently in that it gradually weakens. Already in the first passage the smallest effective dose for guinea-pigs, rats, and mice is larger than for rabbits, and that applied to all the methods of infection.

In guinea-pigs also the period of incubation in the first passage by any method of infection, at least with the higher dilutions, is generally longer than in rabbits.

In guinea-pigs the possibility of intra-peritoneal infection with diminishing doses of the virus quickly disappears. Three of the strains could not be transmitted in that way, and with only two strains could the disease be transmitted for the third passage.

Whether the strains were of a higher or a lower virulence and whether they had undergone few or many passages in guinea-pigs their behaviour was fundamentally always the same. The attenuation of the virulence of the strains occurred slower or quicker, but they always, and by all the methods of infection, eventually completely lost their virulence.

This is a fundamental difference between the virus of Aujeszky's disease and the virus of rabies. Whereas the former in unlimited passages in rabbits shows unaltered virulence and incubation period, in guinea-pigs and rats they more or less quickly become weakened; with the street virus of rabies the virulence in passages through guinea-pigs remains the same or slowly increases with gradual shortening of the period of incubation. In passage experiments with guinea-pigs and rats the virulence of the street strain of virus in general increases more quickly and to a higher degree than in rabbits. The fixed virus is not increased in virulence or in the period of incubation by passage in rabbits. In guinea-pigs, on the other hand, the virulence increases considerably with continued passages. The street strains of virus are in general virulent by any method of infection; with long continued rabbit passages by subcutaneous inoculation they generally lose their virulence and frequently also the susceptibility to intra-muscular transmission, while the Aujeszky strains remain virulent for an unlimited time.

Feeding Infection.—Of 27 feeding experiments nine had a positive result. Of 65 feeding experiments with the smaller animals, in which virulent material was administered once or several times, 15 contracted the disease, namely three of five rabbits, six of 36 rats, and seven of 24 guinea-pigs. In these experiments the virus was given mixed with the food and not through an œsophageal tube, in order to avoid the infliction of wounds of the mucous membrane.

In the experiments with Aujeszky's virus the attempts to transmit the disease through food containing the virus was only to a very limited extent successful. The majority of the rabbits, guinea-pigs and rats which had once or repeatedly been fed with gross quantities of virus remained healthy. The animals that were clinically healthy even after having been thus fed for a long time did not acquire any immunity, even against weak re-infection.

In numerous experiments of the same kind with pigs it was found to be impossible to transmit the disease to them.

Since feeding of the virus of Aujeszky is seldom infective, there appears to be little chance that the disease is naturally transmitted in that way or that healthy animals become infected by eating the carcases of animals dead from the disease.

Infection by Contact.—When diseased animals, whether rabbits, guinea-pigs or rats, are put into the same cage with healthy animals, in which experiments care must be taken that the sound animals are not infected by the infliction of wounds and the carcases of the dead animals are removed intact at the earliest possible moment, it not rarely happens that the experiment has a positive result. Such contact transmission occasionally occurs even when the infected animals are removed from the cage immediately after the outbreak of the disease. On two occasions the disease was transmitted to the sound animals even when the infected animals, before the outbreak of the disease and in clinically sound condition, had been removed from the cage.

Of 83 animals which had been kept in contact with animals suffering from Aujeszky's disease or in the incubative stage, 13 (15·6 per cent.) became infected, namely of 18 rabbits four, of 28 rats four, and of 33 guinea-pigs five. This was considered an important addition to the evidence regarding the natural transmission of Aujeszky's disease. The

disease can be transmitted from diseased to healthy animals without the latter having experienced a wound and without having been infected through bites or eating of infected carcases. It is admitted, however, that the exact way, especially the point of entrance of the virus in natural infection, has not yet been determined. It is not yet known with certainty what the method is or whether it is always the same. The authors hold that, in any case, they have for the first time shown the possibility of the transmission of Aujeszky's disease by simple contact.

Distribution of the Virus in the Diseased Organs and its Excretion by the Diseased Animals.—In the experiments bearing on these questions the animals were killed by puncture of the heart, and the blood thus obtained was immediately injected in ½ c.c. amounts into the muscles. The other organs were immediately removed and made into a thick emulsion with common salt, which in doses of 1 c.c. was injected to the brain.

The results showed that the Au. D. virus, no matter which strain and what method of infection was employed, was spread throughout the whole body and in all the organs more or less uniformly. Since the virus is quite frequently present in the blood is may be assumed that the various organs are infected by way of the blood stream. When such experiments were carried out with the rabbit virus it appeared to be indifferent which animal species was used for the experiments. Most frequently the virus was detected in the spleen, adrenals, salivary glands, and pancreas; more rarely in the liver, kidneys, sexual organs, and lungs.

The results were different when guinea-pigs were employed for the virus passages. In that case the different strains more or less rapidly disappeared from the blood and organs. It also appeared that the virus persisted longer in the adrenals, spleen, pancreas and salivary glands than in the other organs examined. It was especially from the liver, lungs and sexual organs that the virus disappeared rather quickly. There appeared to be no recognisable connection between the virulence of the strains and their occurrence in the different organs.

Experiments with Saliva.—In previous experiments other authors who have endeavoured to discover whether the saliva of animals affected with Aujesky's disease is virulent, that is, whether it is possible that in natural circumstances the disease can be transmitted by biting, have either left the question undecided or, like Remlinger and Bailly, have obtained negative results. It is difficult to obtain saliva from animals affected with this disease. Small animals, with the exception of rabbits, often do not salivate. In the ox, and often also in the pig, there is a strong secretion of saliva, but owing to the restlessness of the animals it is difficult to collect. To the present authors it appeared probable that the saliva would contain the virus, which they had regularly found in the salivary glands in their previous experiments with the different organs. In one of the contact experiments it was unexpectedly found that the disease was transmitted by a bite from one small animal to another. Further experiments showed that,. at least occasionally, the saliva of animals affected with the disease contained the virus.

Recognition of the Virus during the Period of Incubation.—Experiments showed that, no matter what strain was used and what manner of infection was employed, in from two to seven days, and in one case ten days, before the appearance of clinical symptoms, the virus was detected in the brain when the animals were killed, generally after an exceptionally

long period of incubation, which was the shorter the nearer the outbreak of the disease lay. Two or three days before the outbreak of the disease the virus could be found also in the other organs. Thus the results further indicated the constant existence of the latent neurotropism of the Au. D. virus, since by any method of infection the brain always contains the virus earlier than the other organs.

In testing animals which had recovered from infection with the virus it was found that the animals had been successfully immunised, and about two weeks after their recovery they were found to be strongly immunised against re-infection.

Infection Experiments with Recovered Animals.—The experiments on this question were so arranged that always one healthy animal was infected with the same dose and at the same time as a recovered animal. Rabbit virus was always used for the re-infection. The experiments were only made when the control, at the proper time and beyond any doubt, became infected.

In all the laboratory animals, within two weeks after clinical recovery from the first attack of Au. D., even after weak re-infection, no immunity could be recognised, even after cerebral infection and also when other methods and doses near the minimal infective amount were used for re-infection.

Animals which have resisted infection with the virus are after 18 days at the earliest immune against any of the preferred methods of re-infection with an Au. D. strain. In the experiments the immunity lasted at least nine months and probably it may last considerably longer. On the other hand, there is never even a suggestion of immunity against the fixed virus or the street virus or rabies. The animals always become infected even with the minimum lethal dose without any lengthening prolongation of the period of incubation. Conversely, animals highly immune against rabies regularly succumb to any method of infection with Aujesky's disease, even when the smallest pathogenic dose is employed.

Results of Serum Experiments.—A monovalent or polyvalent obtained by hyper-immunising animals that had recovered from inoculation with Aujeszky's virus was fatal to all the strains of that virus even in the strongest concentration in test tubes. It is, therefore, impossible to neutralise it even with the smallest minimum lethal dose of fixed virus or of street virus strains. Conversely rabies immune sera never killed any of the Au. D. strains even in the smallest pathogenic dose. The result of this experiment, therefore, makes the view of Nicolic, that the Aujeszky virus is a variety of the rabies virus, completely untenable. Between the two viruses there is not the slightest immunological or serological relationship.

Summary.

(1) The virus of Aujeszky's disease is a latent neurotropic filtrable virus which stands near the virus of rabies but in many fundamental points is different.

(2) Against desiccation and chemical agents the Aujeszky virus behaves like the rabies virus but appears in general to be rather less resistant.

(3) The eight strains of the Aujeszky virus examined by the authors showed in respect of virulence and period of incubation some considerable

differences. They were, however, all able to produce different clinical pictures.

(4) All the usual experimental animals can easily be infected experimentally with the Aujeszky virus.

(5) The transmission of Aujeszky's disease by feeding succeeded only exceptionally in infecting the experimental animals.

(6) The authors were the first to succeed in transmitting the disease from diseased to healthy animals by simple contact. Exceptionally this occurred even when the infected animal was separated from the healthy animal while the former was still in the incubation period.

(7) Occasionally Aujeszky's disease may be transmitted by biting.

(8) In pronounced cases of the disease the parenchymatous organs and the blood contain the virus. It is regularly present in the blood and in the organs occasionally some days before the symptoms of infection can be detected.

(9) In the urine and fæces the virus is not recognisable. On the other hand, for the first time the authors were successful in transmitting Aujeszky's disease with saliva from a diseased bovine anmal. In attempts to transmit the disease with straw from cages and soiled food the presence of the virus could never be detected.

(10) The strains of Aujeszky's disease virus can always be transmitted in rabbits without change in virulence or length of the period of incubation. In guinea-pigs and rats the virus gradually declines in virulence by repeated passage, eventually so far that the infective power for the animals is lost. The loss of virus thus brought about can be restored by a single passage in a rabbit. This happened with all the strains of the Au. D. viruses in the same way although the number of passages necessary to destroy the virulence was very different in the different cases.

(11) The authors did not beyond doubt succeed in immunising healthy experimental animals against Au. D. Animals which have recovered from the disease show soon after their recovery a high degree of immunity against re-infection for at least nine months and probably for a much longer period. Nevertheless, the serum of these animals is virucidal only to a moderate degree. Strong subcutaneous or intraperitoneal immunisation is very well borne. In this way one can obtain a very strong anti-virus serum.

(12) Animals treated in this way show a high degree of immunity against a concentrated cerebral re-infection with all the tested strains of Aujeszky's disease. On the contrary these showed not the slightest immunity against rabies infection.

(13) The described methods of obtaining the anti-virus serum are strictly specific. They are fatal to all the tested Au. D. viruses even in the strongest concentration, but are unable to neutralise the virus of rabies, even in the weakest dilutions.

(14) Conversely, animals immune to rabies are not protected even against a weak re-infection with the Au. D. virus. Rabies sera are not fatal to the Au. D. virus.

Printed in Great Britain by H. R. Grubb, Ltd., Croydon.

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